

see listing A

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JCS03 U.S. PTO

PATENT
Attorney Docket No. DIVER1180-1

JCS15 U.S. PTO
09/382242
08/24/99

- ☐ NEW PATENT APPLICATION
- ☐ CONTINUATION-IN-PART
- ☒ CONTINUATION
- ☐ DIVISIONAL
- ☐ FILE WRAPPER CONTINUATION

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Rita H. Jennings
(SIGNATURE OF PERSON MAILING PAPER OR FEE)

Sir:

Transmitted herewith for filing is the divisional patent application of

Inventors: Dan E. Robertson; Dennis Murphy; John Reid; Anthony M. Maffia; Steven Link;
Ronald V. Swanson; Patrick V. Warren

For: **ESTERASES**

This is a request for filing a X continuation ☐ divisional application under 37 C.F.R. 1.53(b), of prior Application No. 08/602,359, filed on February 16, 1996, now pending.

FULL NAME OF FIRST INVENTOR	LAST NAME: Robertson	FIRST NAME: Dan	MIDDLE NAME: E.
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 33 Evergreen Lane	CITY AND STATE: Haddonfield, New Jersey	ZIP CODE: 08033
FULL NAME OF SECOND INVENTOR	LAST NAME: Murphy	FIRST NAME: Dennis	MIDDLE NAME: none
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 10 Fairway Road	CITY AND STATE: Paoli, Pennsylvania	ZIP CODE: 19301

FULL NAME OF THIRD INVENTOR	LAST NAME: Reid	FIRST NAME: John	MIDDLE NAME: none
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 922 Montgomery Avenue, Apt. J-2	CITY AND STATE: Bryn Mawr, Pennsylvania	ZIP CODE: 19010
FULL NAME OF FOURTH INVENTOR	LAST NAME: Maffia	FIRST NAME: Anthony	MIDDLE NAME: M.
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 2505-3A Cedar Tree Lane	CITY AND STATE: Wilmington, Delaware	ZIP CODE: 19810
FULL NAME OF FIFTH INVENTOR	LAST NAME: Link	FIRST NAME: Steven	MIDDLE NAME: none
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 108 Gibson Avenue	CITY AND STATE: Wilmington, Delaware	ZIP CODE: 19803
FULL NAME OF SIXTH INVENTOR	LAST NAME: Swanson	FIRST NAME: Ronald	MIDDLE NAME: V.
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 309 N. Lemon Street, Apt. A	CITY AND STATE: Media, Pennsylvania	ZIP CODE: 19063
FULL NAME OF SEVENTH INVENTOR	LAST NAME: Warren	FIRST NAME: Patrick	MIDDLE NAME: V.
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: Sheffield Ave.	CITY AND STATE: Philadelphia, Pennsylvania	ZIP CODE: 19163
FULL NAME OF EIGHTH INVENTOR	LAST NAME: Kosmotka	FIRST NAME: Anna	MIDDLE NAME: none
CITIZENSHIP	STATE OR FOREIGN COUNTRY: USA		
POST OFFICE ADDRESS	POST OFFICE ADDRESS: 224 Harrison Road	CITY AND STATE: Brookhaven, Pennsylvania	ZIP CODE: 19015

No payment of the issue fee, abandonment of, or termination of proceeding has occurred in the above-identified prior application.

1. ☒ Cancel in this application original claims 2-20 of the prior application. (At least one original independent claim must be retained for filing purposes.)
2. ☒ A preliminary amendment is enclosed.

The filing fee has been calculated as shown below:

For	Number Filed		Number Extra		Rate			Fee	
					Small Entity	Other Entity		Small Entity	Other Entity
Total Claims	6	=	0	X	\$9	\$18	=	\$.00	\$ 0
Independent Claims	1	=	0	X	\$39	\$78	=	\$.00	0
Multiple Dependent Claims Presented: ___ Yes <u>X</u> No					\$130	\$260			0
BASIC FEE					\$380	\$760		\$380.00	\$ 0
					TOTAL FEE			\$380.00	\$ 0

3. X Please charge my Deposit Account No. 07-1895 the TOTAL FEE of \$380.00, which covers the filing fee for this application. A duplicate copy of this sheet is enclosed.
4. X The Assistant Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 07-1895. A duplicate copy of this sheet is enclosed.
 - X Any additional filing fees required under 37 C.F.R. 1.16.
 - X Any patent application processing fees under 37 C.F.R. 1.17.
5. X Amend the specification by inserting before the first paragraph on page 1:

This application is a X continuation ___ divisional of application
Serial No. 08/602,359 filed on February 16, 1996, now pending; the entire
contents of which are hereby incorporated by reference herein.
6. X A verified statement claiming small entity status was filed in parent application
Serial No. 08/602,359, filed July 25, 1996, and such status is still proper.
7. X The prior application is assigned of record to RECOMBINANT BIOCATALYSIS,
INC.
8. X The power of attorney in the prior application is to Lisa A. Haile, Registration
No. 38,347.
9. X Please transfer the drawings from the prior application to the new application.

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10. X A true copy of the prior application as filed is enclosed, including the Declaration and Power of Attorney filed in parent application, U.S. Serial No. 08/602,359, filed February 16, 1996.
11. X An Associate Power of Attorney is enclosed.
12. — Information Disclosure Statements filed in the prior application under 37 C.F.R. 1.97 are hereby made of record.
13. X Please transfer the computer readable form (CRF) copy of the Sequence Listing from the prior application, which CRF copy was filed with a Communication mailed July 28, 1997, to this new application.
14. X Please transfer the Statement under 37 C.F.R. § 1.821(f) and (g) from the prior application, which Statement was filed with a Communication mailed July 28, 1997, to this new application.
15. — Also enclosed: Copy of Petition for Extension of Time in parent application U.S. Serial No.: _____

Address all future communications to:

Lisa A. Haile, Ph.D.
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1600
San Diego, California 92121-2189
Telephone: 858-677-1456
Facsimile: 858-677-1465

The undersigned states that the enclosed application papers comprise a true copy of the prior application as filed.

Respectfully submitted,

Date: August 24, 1999

Lisa A. Haile, Ph.D.
Attorney for Applicant
Registration No. 38,347

GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1600
San Diego, CA 92121-2189
GT6143399
104703-156621

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:) Group Art Unit: (Unassigned)
Robertson et al.)
) Examiner: (Unassigned)
Filed: Herewith)
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Parent Serial No.: 08/602,359)
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Parent Filing Date: February 16, 1996)
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For: ESTERASES)
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Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

This Preliminary Amendment is being filed herewith further to a request under 37 C.F.R. § 1.53(b) to file a continuation application based on Application Serial No. 08/602,359, filed February 16, 1996, now pending.

Please cancel claim 1 of the application, and add new claims 21-26 as follows:

--21. (New) An oligonucleotide probe consisting of at least about 15 contiguous nucleotides of a polynucleotide selected from the group consisting of SEQ ID NO:23-31 and SEQ ID NO:32.

22. (New) An oligonucleotide probe fully complementary to an oligonucleotide probe of Claim 21.

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23. (New) The oligonucleotide probe of claims 21 or 22 wherein the probe is 20-50 nucleotides in length.

24. (New) The oligonucleotide probe of claims 21 or 22 wherein the probe is labeled with a detectable label.

25. (New) The oligonucleotide probe of claim 24, wherein the detectable label is an isotopic label or a non-isotopic label, which non-isotopic label is selected from the group consisting of: a fluorescent molecule, a chemiluminescent molecule, an enzyme, a cofactor, an enzyme substrate, and a hapten.

26. (New) The oligonucleotide probe of Claim 24, wherein the probe comprises a sequence which specifically hybridizes to a nucleic acid comprising SEQ ID NO:23-32 or a sequence fully complementary thereto to form a detectable target probe duplex.--

Remarks

By the present communication, new claims 21-26 have been added. No new matter is introduced by the new claim language, as the newly presented claims are fully supported by Applicant's specification and original claims. Accordingly, claims 21-26 are currently pending.

It is believed that the application is in condition for allowance and, therefore, prompt and favorable action is earnestly solicited. If there are any questions concerning this communication, the Examiner is invited to call the undersigned at the telephone number provided below.

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No fee is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any fee is required, authorization is given to charge the amount of this fee to Deposit Account No. 07-1895.

Respectfully submitted,

Date: August 24, 1999

Lisa A. Haile, Ph.D.

Telephone: (858) 677-1456

Facsimile: (858) 677-1465

GRAY, CARY, WARE & FREIDENRICH, LLP
4365 Executive Drive, Suite 1600
San Diego, California 92121-2189

ESTERASES

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production and isolation of such polynucleotides and polypeptides. More particularly, the polynucleotides and polypeptides of the present invention have been putatively identified as esterases. Esterases are enzymes that catalyze the hydrolysis of ester groups to organic acids and alcohols.

Many esterases are known and have been discovered in a broad variety of organisms, including bacteria, yeast and higher animals and plants. A principal example of esterases are the lipases, which are used in the hydrolysis of lipids, acidolysis(replacement of an esterified fatty acid with a free fatty acid) reactions, transesterification(exchange of fatty acids between triglycerides)reactions, and in ester synthesis. The major industrial applications for lipases include: the detergent industry, where they are employed to decompose fatty materials in laundry stains into easily removable hydrophilic substances; the food and beverage industry where they are used in the manufacture of cheese, the ripening and flavoring of cheese, as antistaling agents for bakery products, and in the production of margarine and other spreads with natural

butter flavors; in waste systems; and in the pharmaceutical industry where they are used as digestive aids.

The polynucleotides and polypeptides of the present invention have been identified as esterases as a result of their enzymatic activity.

In accordance with one aspect of the present invention, there are provided novel enzymes, as well as active fragments, analogs and derivatives thereof.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding the enzymes of the present invention including mRNAs, cDNAs, genomic DNAs as well as active analogs and fragments of such enzymes.

In accordance with another aspect of the present invention there are provided isolated nucleic acid molecules encoding mature polypeptides expressed by the DNA contained in ATCC Deposit No. _____.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence of the present invention, under conditions promoting expression of said enzymes and subsequent recovery of said enzymes.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes for hydrolyzing ester groups to yield an organic acid and an alcohol. The esterases of the invention are stable at high temperatures and in organic solvents and, thus, are superior for use in production of optically pure chiral compounds used in pharmaceutical, agricultural and other chemical industries.

In accordance with yet a further aspect of the present invention, there are also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to hybridize to a nucleic acid sequence of the present invention.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes, for *in vitro* purposes related to scientific research, for example, to generate probes for identifying similar sequences which might encode similar enzymes from other organisms by using certain regions, *i.e.*, conserved sequence regions, of the nucleotide sequence.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 is an illustration of the full-length DNA (SEQ ID NO:23) and corresponding deduced amino acid sequence (SEQ ID NO:33) of *Staphylothermus marinus* F1-12LC of the present invention. Sequencing was performed using a 378 automated DNA sequencer (Applied Biosystems, Inc.) for all sequences of the present invention.

Figure 2 is an illustration of the full-length DNA (SEQ ID NO:24) and corresponding deduced amino acid sequence (SEQ ID NO:34) of *Pyrodictium* TAG11-17LC.

Figure 3 is an illustration of the full-length DNA (SEQ ID NO:25) and corresponding deduced amino acid sequence (SEQ ID NO:35) of *Archaeoglobus venificus* SNP6-24LC.

Figure 4 is an illustration of the full-length DNA (SEQ ID NO:26) and corresponding deduced amino acid sequence (SEQ ID NO:36) of *Aquifex pyrophilus*-28LC.

Figure 5 is an illustration of the full-length DNA (SEQ ID NO:27) and corresponding deduced amino acid sequence (SEQ ID NO:37) of M11TL-29L.

Figure 6 is an illustration of the full-length DNA (SEQ ID NO:28) and corresponding deduced amino acid sequence (SEQ ID NO:38) of *Thermococcus* CL-2-30LC.

Figure 7 is an illustration of the full-length DNA (SEQ ID NO:29) and corresponding deduced amino acid sequence (SEQ ID NO:39) of *Aquifex* VF5-34LC.

Figure 8 is an illustration of the full-length DNA (SEQ ID NO:30) and corresponding deduced amino acid sequence (SEQ ID NO:40) of *Teredinibacter*-42L.

Figure 9 is an illustration of the full-length DNA (SEQ ID NO:31) and corresponding deduced amino acid sequence (SEQ ID NO:41) of *Archaeoglobus fulgidus* VC16-16MC.

Figure 10 is an illustration of the full-length DNA (SEQ ID NO:32) and corresponding deduced amino acid sequence (SEQ ID NO:42) of *Sulfolobus solfataricus* P1-8LC.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA, which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences ultimately process to produce the desired protein.

"Recombinant" enzymes refer to enzymes produced by recombinant DNA techniques; *i.e.*, produced from cells transformed by an exogenous DNA construct encoding the desired enzyme. "Synthetic" enzymes are those prepared by chemical synthesis.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular enzyme, is a DNA sequence which is transcribed and translated into an enzyme when placed under the control of appropriate regulatory sequences.

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode for the mature enzymes having the deduced amino acid sequences of Figures 1-10 (SEQ ID NOS:23-32).

In accordance with another aspect of the present invention, there are provided isolated polynucleotides encoding the enzymes of the present invention. The deposited material is a mixture of genomic clones comprising DNA encoding an enzyme of the present invention. Each genomic clone comprising the respective DNA has been inserted into a pBluescript vector (Stratagene, La Jolla, CA). The deposit has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA, on December 13, 1995 and assigned ATCC Deposit No. _____.

The deposit(s) have been made under the terms of the Budapest Treaty on the

International Recognition of the deposit of micro-organisms for purposes of patent procedure. The strains will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit would be required under 35 U.S.C. §112. The sequences of the polynucleotides contained in the deposited materials, as well as the amino acid sequences of the polypeptides encoded thereby, are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The polynucleotides of this invention were originally recovered from genomic gene libraries derived from the following organisms:

Staphylothermus marinus F1 is a thermophilic sulfur archaea which was isolated in Vulcano, Italy. It grows optimally at 85°C ($T_{\max} = 98^{\circ}\text{C}$) at pH 6.5.

Pyrodictium TAG11 is a thermophilic sulfur archaea which was isolated in the Middle Atlantic Ridge. It grows optimally at 103°C ($T_{\max} = 110^{\circ}\text{C}$) at pH 6.5.

Archaeoglobus venificus SNP6 was isolated in the Middle Atlantic Ridge and grows optimally at 75°C ($T_{\max} = 92^{\circ}\text{C}$) at pH 6.9.

Aquifex pyrophilus K01 5a was isolated at Kolbeinsey Ridge, North of Iceland. This marine organism is a gram-negative, rod-shaped, strictly chemolithoautrophic, knall gas bacterium. It grows optimally at 85°C ($T_{\max} = 95^{\circ}\text{C}$) at pH 6.8.

M11TL is a new species of *Desulfurococcus* which was isolated from Diamond Pool (formerly Jim's Black Pool) in Yellowstone. The organism grows heterotrophically by fermentation of different organic materials (sulfur is not necessary)

in grape-like aggregates optimally at 85 - 88°C in a low salt medium at pH 7.0.

Thermococcus CL-2 was isolated in the North Cleft Segment of the Juan de Fuca Ridge from a severed alvinellid worm residing on a "black smoker" sulfide structure. This marine archaea forms pleomorphic cocci, and grows optimally at 88°C.

Aquifex VF5 was isolated at a beach in Vulcano, Italy. This marine organism is a gram-negative, rod-shaped, strictly chemolithoautotrophic, knall gas bacterium. It grows optimally at 85°C ($T_{\max} = 95^{\circ}\text{C}$) at pH 6.8.

Teredinibacter (pure) is an endosymbiont of the shipworm *Bankia gouldi*. The organism has straight to slightly bent 5-10 μm rods, and forms spiral cells as stationary phase is met. The organism was described in Science (1983) 22:1401-1403. It grows optimally at 30°C at pH 8.0.

Archaeoglobus fulgidus VC16 was isolated in Vulcano, Italy. The organism grows optimally at 85°C ($T_{\max} = 92^{\circ}\text{C}$) at pH 7.0.

Sulfolobus solfataricus P1 grows optimally at 85°C ($T_{\max} = 87^{\circ}\text{C}$) at pH 2.0.

Accordingly, the polynucleotides and enzymes encoded thereby are identified by the organism from which they were isolated, and are sometimes hereinafter referred to as F1/12LC (Figure 1 and SEQ ID NOS:23 and 33), TAG11/17LC (Figure 2 and SEQ ID NOS:24 and 34), SNP6/24LC (Figure 3 and SEQ ID NOS:25 and 35), AqP/28LC (Figure 4 and SEQ ID NOS:26 and 36), M11TL/29L (Figure 5 and SEQ ID NOS:27 and 37), CL-2/30LC (Figure 6 and SEQ ID NOS:28 and 38), VF5/34LC (Figure 7 and SEQ ID NOS:29 and 39), Trb/42L (Figure 8 and SEQ ID NOS:30 and 40), VC16/16MC (Figure 9 and SEQ ID NOS:31 and 41) and P1/8LC (Figure 10 and SEQ ID NOS: 32 and 42).

The polynucleotides and polypeptides of the present invention show identity at the nucleotide and protein level to known genes and proteins encoded thereby as shown in Table 1.

Table 1

Enzyme	Gene w/closest Homology (Organism)	Protein Similarity (%)	Protein Identity (%)	DNA Identity (%)
F1/12LC	No significant homology	-	-	-
TAG11/17LC	No significant homology	-	-	-
SNP6/24LC	PIR S34609 - carboxylesterase <i>Pseudomonas</i> sp. (strain KWI-56) open reading frame of unknown function in <i>E.coli</i> .	46	27	42
AqP/29LC		53	31	38
M11TL/29LC	No significant homology	-	-	-
CL02/30LC	No significant homology	-	-	-
VF5/34LC	Identified by homology to 28LC; also homologous to ORF of unknown function 5' of tgs in <i>E. coli</i>	84	71	71
Trb/42L	No significant homology	-	-	-
P1-8LC				
VC16-16MC				

All the clones identified in Table 1 encode polypeptides which have esterase activity.

This invention, in addition to the isolated nucleic acid molecules encoding the enzymes of the present invention, also provides substantially similar sequences. Isolated nucleic acid sequences are substantially similar if: (i) they are capable of hybridizing under conditions hereinafter described, to the polynucleotides of SEQ ID NOS:23-32; (ii) or they encode DNA sequences which are degenerate to the polynucleotides of SEQ ID NOS:23-32. Degenerate DNA sequences encode the amino acid sequences of SEQ ID NOS:33-42, but have variations in the nucleotide coding sequences. As used herein, substantially similar refers to the sequences having similar identity to the sequences of the instant invention. The nucleotide sequences that are substantially the same can be identified by hybridization or by sequence comparison. Enzyme sequences that are substantially the same can be identified by one or more of the following: proteolytic digestion, gel electrophoresis and/or microsequencing.

One means for isolating the nucleic acid molecules encoding the enzymes of the present invention is to probe a gene library with a natural or artificially designed probe using art recognized procedures (see, for example: Current Protocols in Molecular Biology, Ausubel F.M. *et al.* (EDS.) Green Publishing Company Assoc. and John Wiley Interscience, New York, 1989, 1992). It is appreciated by one skilled in the art that the polynucleotides of SEQ ID NOS:23-32, or fragments thereof (comprising at least 12 contiguous nucleotides), are particularly useful probes. Other particularly useful probes for this purpose are hybridizable fragments of the sequences of SEQ ID NOS:1-22 (*i.e.*, comprising at least 12 contiguous nucleotides).

With respect to nucleic acid sequences which hybridize to specific nucleic acid sequences disclosed herein, hybridization may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions. As an example of oligonucleotide hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 0.5% SDS, 10X Denhardt's, and 0.5 mg/mL polyriboadenylic acid. Approximately 2 X 10⁷ cpm (specific activity 4-

9 X 10⁸ cpm/ug) of ³²P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m -10°C for the oligo-nucleotide probe. The membrane is then exposed to autoradiographic film for detection of hybridization signals.

Stringent conditions means hybridization will occur only if there is at least 90% identity, preferably at least 95% identity and most preferably at least 97% identity between the sequences. See J. Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual, 2d Ed.*, Cold Spring Harbor Laboratory (1989) which is hereby incorporated by reference in its entirety.

As used herein, a first DNA (RNA) sequence is at least 70% and preferably at least 80% identical to another DNA (RNA) sequence if there is at least 70% and preferably at least a 80% or 90% identity, respectively, between the bases of the first sequence and the bases of the another sequence, when properly aligned with each other, for example when aligned by BLASTN.

The present invention relates to polynucleotides which differ from the reference polynucleotide such that the changes are silent changes, for example the change do not alter the amino acid sequence encoded by the polynucleotide. The present invention also relates to nucleotide changes which result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference polynucleotide. In a preferred aspect of the invention these polypeptides retain the same biological action as the polypeptide encoded by the reference polynucleotide.

The polynucleotides of this invention were recovered from genomic gene libraries from the organisms listed in Table 1. Gene libraries were generated in the Lambda ZAP II cloning vector (Stratagene Cloning Systems). Mass excisions were

performed on these libraries to generate libraries in the pBluescript phagemid. Libraries were generated and excisions were performed according to the protocols/methods hereinafter described.

The polynucleotides of the present invention may be in the form of RNA or DNA which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequences which encodes the mature enzymes may be identical to the coding sequences shown in Figures 1-10 (SEQ ID NOS:23-32) or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature enzymes as the DNA of Figures 1-10 (SEQ ID NOS:23-32).

The polynucleotide which encodes for the mature enzyme of Figures 1-10 (SEQ ID NOS:33-42) may include, but is not limited to: only the coding sequence for the mature enzyme; the coding sequence for the mature enzyme and additional coding sequence such as a leader sequence or a proprotein sequence; the coding sequence for the mature enzyme (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature enzyme.

Thus, the term "polynucleotide encoding an enzyme (protein)" encompasses a polynucleotide which includes only coding sequence for the enzyme as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the enzymes having the deduced amino acid sequences of Figures 1-10 (SEQ ID NOS:33-42). The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature enzymes as shown in Figures 1-10 (SEQ ID NOS:23-32) as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the enzymes of Figures 1-10 (SEQ ID NOS:23-32). Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequences shown in Figures 1-10 (SEQ ID NOS:23-32). As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded enzyme.

Fragments of the full length gene of the present invention may be used as hybridization probes for a cDNA or a genomic library to isolate the full length DNA and to isolate other DNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 10, preferably at least 15, and even more preferably at least 30 bases and may contain, for example, at least 50 or more bases. The probe may also be used to identify a DNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of genomic DNA to determine which members of the library the probe hybridizes to.

It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. The probes are thus useful to isolate

complementary copies of DNA from other sources or to screen such sources for related sequences.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode enzymes which either retain substantially the same biological function or activity as the mature enzyme encoded by the DNA of Figures 1-10 (SEQ ID NOS:23-32).

Alternatively, the polynucleotide may have at least 15 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to any part of a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the polynucleotides of SEQ ID NOS:23-32, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% identity and more preferably at least a 95% identity to a polynucleotide which encodes the enzymes of SEQ ID NOS:33-42 as well as fragments thereof, which fragments have at least 15 bases, preferably at least 30 bases and most preferably at least 50 bases, which fragments are at least 90% identical, preferably at least 95% identical and most preferably at least 97% identical under stringent conditions to any portion of a polynucleotide of the present invention.

The present invention further relates to enzymes which have the deduced amino

acid sequences of Figures 1-10 (SEQ ID NOS:23-32) as well as fragments, analogs and derivatives of such enzyme.

The terms "fragment," "derivative" and "analog" when referring to the enzymes of Figures 1-10 (SEQ ID NOS:33-42) mean enzymes which retain essentially the same biological function or activity as such enzymes. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature enzyme.

The enzymes of the present invention may be a recombinant enzyme, a natural enzyme or a synthetic enzyme, preferably a recombinant enzyme.

The fragment, derivative or analog of the enzymes of Figures 1-10 (SEQ ID NOS:33-42) may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature enzyme is fused with another compound, such as a compound to increase the half-life of the enzyme (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature enzyme, such as a leader or secretory sequence or a sequence which is employed for purification of the mature enzyme or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The enzymes and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a

naturally-occurring polynucleotide or enzyme present in a living animal is not isolated, but the same polynucleotide or enzyme, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or enzymes could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The enzymes of the present invention include the enzymes of SEQ ID NOS:33-42 (in particular the mature enzyme) as well as enzymes which have at least 70% similarity (preferably at least 70% identity) to the enzymes of SEQ ID NOS:33-42 and more preferably at least 90% similarity (more preferably at least 90% identity) to the enzymes of SEQ ID NOS:33-42 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the enzymes of SEQ ID NOS:33-42 and also include portions of such enzymes with such portion of the enzyme generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two enzymes is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one enzyme to the sequence of a second enzyme.

A variant, *i.e.* a "fragment", "analog" or "derivative" polypeptide, and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the

aromatic residues Phe, Tyr.

Most highly preferred are variants which retain the same biological function and activity as the reference polypeptide from which it varies.

Fragments or portions of the enzymes of the present invention may be employed for producing the corresponding full-length enzyme by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length enzymes. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of enzymes of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, *etc.* The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing enzymes by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing an enzyme. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, *e.g.*, derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as

vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli. lac* or *trp*, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses.

The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, *Streptomyces*, *Bacillus subtilis*; fungal cells, such as yeast; insect cells such as *Drosophila S2* and *Spodoptera Sf9*; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, *etc.* The selection of an

appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example; Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBluescript II KS, ptrc99a, pKK223-3, pDR540, pRIT2T (Pharmacia); Eukaryotic: pXT1, pSG5 (Stratagene) pSVK3, pBPV, pMSG, pSVL, SV40 (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., *Basic Methods in*

Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the enzymes of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the enzymes of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence

capable of directing secretion of translated enzyme. Optionally, the heterologous sequence can encode a fusion enzyme including an N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell*, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The enzyme can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The enzymes of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the enzymes of the present invention may be glycosylated or may be non-glycosylated. Enzymes of the invention may or may not also include an initial methionine amino acid residue.

Esterases are a group of key enzymes in the metabolism of fats and are found in all organisms from microbes to mammals. In the hydrolysis reaction, an ester group is hydrolysed to an organic acid and an alcohol.

Esterases enantiomerically differentiate dicarboxylic diesters and diacetates of diols. Using the approach disclosed in a commonly assigned, copending provisional application Serial No. 60/008,316, filed on December 7, 1995 and entitled "Combinatorial Enzyme Development," the disclosure of which is incorporated herein by reference in its entirety, one could convert the enantiospecificity of the esterase. Further, the thermostable esterases are believed to have superior stability at higher temperatures and in organic solvents. Thus, they are better suited for use in rigorous production processes which require robust catalysts.

There are a number of industrial and scientific applications for esterases, such as those of the present invention, including:

- 1) Esterases are useful in the dairy industry as ripening starters for cheeses, such as the Swiss-type cheeses;
- 2) Esterases are useful in the pulp and paper industry for lignin removal from cellulose pulps, for lignin solubilization by cleaving the ester linkages between aromatic acids and lignin and between lignin and hemicelluloses, and for disruption of cell wall structure when used in combination with xylanase and other xylan-degrading enzymes in biopulping and biobleaching of pulps;
- 3) Esterases are useful in the synthesis of carbohydrate derivatives, such as sugar derivatives;
- 4) Esterases are useful, when combined with xylanases and cellulases, in the

conversion of lignocellulosic wastes to fermentable sugars for producing a variety of chemicals and fuels;

5) Esterases are useful as research reagents in studies on plant cell wall structure, particularly the nature of covalent bonds between lignin and carbohydrate polymers in the cell wall matrix;

6) Esterases are also useful as research reagents in studies on mechanisms related to disease resistance in plants and the process of organic matter decomposition; and

7) Esterases are useful in selection of plants bred for production of highly digestible animal feeds, particularly for ruminant animals.

Antibodies generated against the enzymes corresponding to a sequence of the present invention can be obtained by direct injection of the enzymes into an animal or by administering the enzymes to an animal, preferably a nonhuman. The antibody so obtained will then bind the enzymes itself. In this manner, even a sequence encoding only a fragment of the enzymes can be used to generate antibodies binding the whole native enzymes. Such antibodies can then be used to isolate the enzyme from cells expressing that enzyme.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, *Nature*, 256:495-497, 1975), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* 4:72, 1983), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic enzyme products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic enzyme products of this invention.

Antibodies generated against an enzyme of the present invention may be used in screening for similar enzymes from other organisms and samples. Such screening techniques are known in the art, for example, one such screening assay is described in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (2d Ed.), Cold Spring Harbor Laboratory, Section 12.21-12.28 (1989) which is hereby incorporated by reference in its entirety.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case "p" preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For

analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel *et al.*, *Nucleic Acids Res.*, 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., *et al.*, *Id.*, p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2d Ed.), Cold Spring Harbor Press (1989).

Example 1

Bacterial Expression and Purification of Esterases

DNA encoding the enzymes of the present invention, SEQ ID NOS:33 through 42, were initially amplified from a pBluescript vector containing the DNA by the PCR technique using the primers noted herein. The amplified sequences were then inserted into the respective PQE vector listed beneath the primer sequences, and the enzyme was expressed according to the protocols set forth herein. The 5' and 3' primer sequences for the respective genes are as follows:

Staphylothermus marinus F1-12LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGTCTTTA AACAAAGCACT CT

3' CGGAAGATCT CTATCGTTTA GTGTATGATT T

vector: pQET

Pyrodictium TAG11-17LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGAACTC CTTGAGCCCA CA

EcoRI

3' CGGAAGATCT CGCCGGTACA CCATCAGCCA C

BglIII

vector: pQET

Archaeoglobus venificus SNP6-24LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGCCATAT GTTAGGAATG GT

3' CGGAGGTACC TTAGAACTGT GCTGAAGAAA TAAATTCGTC CATTGCTCT

3' CGGAGGTACC TTAGAACTGT GCTGAAGAAA TAAATTCGTC CATTGCTCTA TTA

vector: pQET

Aquifex pyrophilus - 28LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGAGATTG AGGAAATTTG AAG

3' CGGAGGTACC CTATTCAGAA AGTACCTCTA A

vector: pQET

M11TL - 29LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGTTTAAT ATCAATGTCT TT

3' CGGAAGATCT TTAAGGATTT TCCCTGGTA G

vector: pQET

Thermococcus CL-2 - 30LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGGAGGTT TACAAGGCCA AA

3' CGGAGGTACC TTATTGAGCC GAAGAGTACG A
vector: pQET

Aquifex VF5 - 34LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAACTATGATTGGC AATTGAAAT TGA EcoRI
3' CGGAGGTACC TTAAAGTGCT CTCATATCCC C KpnI
vector: pQET

Teredinibacter 42L

5' CCGAGAATTC ATTAAAGAGG AGAAATTAACTATGCCAGCT AATGACTCAC CC
3' CGGAAGATCT TCAACAGGCT CCAATAATT TC (without His-tag)
3' CGGAAGATCT ACAGGCTCCA AATAATTTC (with His-tag)
vector: pQE12

Archaeoglobus fulgidus VC16-16MC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAACTATGCTTGAT ATGCCAATCG AC EcoRI
3' CGGAGGTACC CTAGTCGAAG ACAACAAGAG C KpnI
vector: pQET

Sulfolobus solfataricus P1-8LC

5' CCGAGAATTC ATTAAAGAGG AGAAATTAACTATGCCCCAG GATCCTAGAA TT EcoRI
3' CGGAGGTACC TTAAATTTTA TCATAAAATA C KpnI
vector: pQET

The restriction enzyme sites indicated correspond to the restriction enzyme sites on the bacterial expression vector indicated for the respective gene (Qiagen, Inc. Chatsworth, CA). The pQE vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites.

The pQE vector was digested with the restriction enzymes indicated. The amplified sequences were ligated into the respective pQE vector and inserted in frame with the sequence encoding for the RBS. The ligation mixture was then used to transform the *E. coli* strain M15/pREP4 (Qiagen, Inc.) by electroporation. M15/pREP4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance

(Kan^r). Transformants were identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture was used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 (O.D.⁶⁰⁰) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation.

The primer sequences set out above may also be employed to isolate the target gene from the deposited material by hybridization techniques described above.

Example 2

Isolation of a Selected Clone from the Deposited Genomic Clones

The two oligonucleotide primers corresponding to the gene of interest are used to amplify the gene from the deposited material. A polymerase chain reaction is carried out in 25 µl of reaction mixture with 0.1 µg of the DNA of the gene of interest. The reaction mixture is 1.5-5 mM MgCl₂, 0.01 % (w/v) gelatin, 20 µM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 1.25 Unit of Taq polymerase. Thirty cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with the Perkin-Elmer Cetus 9600 thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the gene of interest by subcloning and sequencing the DNA product.

Example 3

Production of the Expression Gene Bank

Colonies containing pBluescript plasmids with random inserts from the organisms M11TL, *Thermococcus* GU5L5, and *Teredinibacter* were obtained according to the method of Hay and Short, *Strategies*, 5:16, 1992.

Example 4

Screening for Lipase/Esterase Activity

The resulting colonies were picked with sterile toothpicks and used to singly inoculate each of the wells of 96-well microtiter plates. The wells contained 250 μ L of LB media with 100 μ g/mL ampicillin, 80 μ g/mL methicillin, and 10% v/v glycerol (LB Amp/Meth, glycerol). The cells were grown overnight at 37°C without shaking. This constituted generation of the "Source GeneBank." Each well of the Source GeneBank thus contained a stock culture of *E. coli* cells, each of which contained a pBluescript with a unique DNA insert.

The plates of the Source GeneBank were used to multiply inoculate a single plate (the "Condensed Plate") containing in each well 200 μ L of LB Amp/Meth, glycerol. This step was performed using the High Density Replicating Tool (HDRT) of the Beckman Biomek with a 1% bleach, water, isopropanol, air-dry sterilization cycle in between each inoculation. Each well of the Condensed Plate thus contained 10 to 12 different pBluescript clones from each of the source library plates. The Condensed Plate was grown for 16 hours at 37°C and then used to inoculate two white 96-well Polyfiltronics microtiter daughter plates containing in each well 250 μ L of LB Amp/Meth (no glycerol). The original condensed plate was put in storage -80°C. The two condensed daughter plates were incubated at 37°C for 18 hours.

The short chain esterase '600 μ M substrate stock solution' was prepared as follows:

25 mg of each of the following compounds was dissolved in the appropriate volume of DMSO to yield a 25.2 mM solution. The compounds used were 4-methylumbelliferyl propionate, 4-methylumbelliferyl butyrate, and 4-methylumbelliferyl heptanoate. Two hundred fifty microliters of each DMSO solution was added to ca 9 mL of 50 mM, pH 7.5 Hepes buffer which contained 0.6% of Triton X-100 and 0.6 mg per mL of dodecyl maltoside (Anatrace). The volume was taken to 10.5 mL with the above Hepes buffer to yield a slightly cloudy suspension.

The long chain '600 μ M substrate stock solution' was prepared as follows: 25 mg of each of the following compounds was dissolved in DMSO to 25.2 mM as above. The compounds used were 4-methylumbelliferyl elaidate, 4-methylumbelliferyl palmitate, 4-methylumbelliferyl oleate, and 4-methylumbelliferyl stearate. All required brief warming in a 70°C bath to achieve dissolution. Two hundred fifty microliters of each DMSO solution was added to the Hepes buffer and diluted to 10.5 mL as above. All seven umbelliferones were obtained from Sigma Chemical Co.

Fifty μ L of the long chain esterase or short chain esterase '600 μ M substrate stock solution' was added to each of the wells of a white condensed plate using the Biomek to yield a final concentration of substrate of about 100 μ M.. The fluorescence values were recorded (excitation = 326 nm, emission = 450 nm) on a plate-reading fluorometer immediately after addition of the substrate. The plate was incubated at 70°C for 60 minutes in the case of the long chain substrates, and 30 minutes at RT in the case of the short chain substrates. The fluorescence values were recorded again. The initial and final fluorescence values were compared to determine if an active clone was present.

Example 5

Isolation and Purification of the Active Clone

To isolate the individual clone which carried the activity, the Source GeneBank plates were thawed and the individual wells used to singly inoculate a new plate containing

LB Amp/Meth. As above, the plate was incubated at 37°C to grow the cells, 50 µL of 600 µM substrate stock solution was added using the Biomek and the fluorescence was determined. Once the active well from the source plate was identified, cells from this active well were streaked on agar with LB/Amp/Meth and grown overnight at 37°C to obtain single colonies. Eight single colonies were picked with a sterile toothpick and used to singly inoculate the wells of a 96-well microtiter plate. The wells contained 250 µL of LB Amp/Meth. The cells were grown overnight at 37°C without shaking. A 200 µL aliquot was removed from each well and assayed with the appropriate long or short chain substrates as above. The most active clone was identified and the remaining 50 µL of culture was used to streak an agar plate with LB/Amp/Meth. Eight single colonies were picked, grown and assayed as above. The most active clone was used to inoculate 3 mL cultures of LB/Amp/Meth, which were grown overnight. The plasmid DNA was isolated from the cultures and utilized for sequencing.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS:

ROBERTSON, Daniel E.

MURPHY, Dennis

REID, John

MAFFIA, Anthony

LINK, Steven

SWANSON, Ronald V.

WARREN, Patrick V.

KOSMOTKA, Anna

(ii) TITLE OF INVENTION:

ESTERASES

(iii) NUMBER OF SEQUENCES: 42

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN,
CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HERRON, CHARLES J.
(B) REGISTRATION NUMBER: 28,019
(C) REFERENCE/DOCKET NUMBER: 331400-39

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 52 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGTCTTTA AACAAGCACT CT

52

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGGAAGATCT CTATCGTTTA GTGTATGATT T

31

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 52 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGAACTC CTTGAGCCCA CA

52

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAAGATCT CGCCGGTACA CCATCAGCCA C

31

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 52 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGAGAATTC ATTAAAGAGG AGAAATTAC TATGCCATAT GTTAGGAATG GT

52

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 53 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGAGGTACC TTAGAACTGT GCTGAAGAA TAAATTCGTC CATTGCTCTA TTA

53

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 49 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGGAGGTACC TTAGAACTGT GCTGAAGAA TAAATTCGTC CATTGCTCT

49

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 53 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCGAGAATTC ATTAAAGAGG AGAAATTAC TATGAGATTG AGGAAATTTG AAG

53

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 31 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGAGGTACC CTATTCAGAA AGTACCTCTA A

31

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 52 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGTTTAAT ATCAATGTCT TT

52

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 31 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGGAAGATCT TTAAGGATT TCCCTGGCTA G

31

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 52 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGGAGGTT TACAAGGCCA AA

52

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 31 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGGAGGTACC TTATTGAGCC GAAGAGTACG A

31

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 53 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCGAGAATTC ATTAAAGAGG AGAAATTAC TATGATTGGC AATTGAAAT TGA

53

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGGAGGTACC TTAAAGTGCT CTCATATCCC C

31

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCGAGAATTC ATTAAAGAGG AGAAATTAC TATGCCAGCT AATGACTCAC CC

52

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 32 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
CGGAAGATCT TCAACAGGCT CCAAATAATT TC 32

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 29 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
CGGAAGATCT ACAGGCTCCA AATAATTTC 29

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 52 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGCTTGAT ATGCCAATCG AC 52

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
CGGAGGTACC CTAGTCGAAC AGAAGAACAG C 31

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 52 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
CCGAGAATTC ATTAAAGAGG AGAAATTAAC TATGCCCTA GATCCTAGAA TT 52

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGAGGTACC TTAAATTTTA TCATAAAATA C

31

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 555 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATG TCT TTA AAC AAG CAC TCT TCG ATG GAT ATG ATA ATA TTT ATT CTC	48
Met Ser Leu Asn Lys His Ser Trp Met Asp Met Ile Ile Phe Ile Leu	
1 5 10 15	
AGC TTT TCT TTC CCA TTA ACA ATG ATC GCA TTA GCT ATC TCT ATG TCG	96
Ser Phe Ser Phe Pro Leu Thr Met Ile Ala Leu Ala Ile Ser Met Ser	
20 25 30	
TCA TGG TTT AAT ATA TGG AAT AAT GCA TTA AGC GAT CTA GGA CAT GCT	144
Ser Trp Phe Asn Ile Trp Asn Asn Ala Leu Ser Asp Leu Gly His Ala	
35 40 45	
GTT AAA AGC AGT GTT GCT CCA ATA TTC AAT CTA GGT CTT GCA ATT GGT	192
Val Lys Ser Ser Val Ala Pro Ile Phe Asn Leu Gly Leu Ala Ile Gly	
50 55 60	
GGG ATA CTA ATT GTT ATA GTT GGT TTA AGA AAT CTT TAT TCG TGG AGT	240
Gly Ile Leu Ile Val Ile Val Gly Leu Arg Asn Leu Tyr Ser Trp Ser	
65 70 75 80	
AGA GTT AAA GGA TCT TTA ATC ATA TCC ATG GGT GTA TTT CTT AAC TTA	288
Arg Val Lys Gly Ser Leu Ile Ile Ser Met Gly Val Phe Leu Asn Leu	
85 90 95	
ATA GGG GTT TTC GAC GAA GTA TAT GGT TGG ATA CAT TTC CTA GTC TCA	336
Ile Gly Val Phe Asp Glu Val Tyr Gly Trp Ile His Phe Leu Val Ser	
100 105 110	
GTA TTG TTT TTC TTA TCA ATA ATA GCA TAT TTC ATA GCT ATA TCA ATA	384
Val Leu Phe Phe Leu Ser Ile Ile Ala Tyr Phe Ile Ala Ile Ser Ile	
115 120 125	
CTT GAC AAA TCA TGG ATA GCT GTT CTA CTA ATA ATA GGT CAT ATT GCA	432
Leu Asp Lys Ser Trp Ile Ala Val Leu Leu Ile Ile Gly His Ile Ala	
130 135 140	
ATG TGG TAT CTA CAC TTT GCT TCA GAG ATT CCG AGA GGT GCT GCT ATT	480

Met	Trp	Tyr	Leu	His	Phe	Ala	Ser	Glu	Ile	Pro	Arg	Gly	Ala	Ala	Ile	
145					150					155					160	
CCC	GAG	TTA	TTA	GCG	GTA	TTC	TCG	TTT	TTA	CCA	TTC	TAT	ATA	AGA	CAG	528
Pro	Glu	Leu	Leu	Ala	Val	Phe	Ser	Phe	Leu	Pro	Phe	Tyr	Ile	Arg	Asp	
				165					170					175		
TAT	TTT	AAA	TCA	TAC	ACT	AAA	CGA	TAG								576
Tyr	Phe	Lys	Ser	Tyr	Thr	Lys	Arg									
				180												

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1041 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATG	AAA	CTC	CTT	GAG	CCC	ACA	AAT	ACC	TCC	TAC	ACG	CTG	TTA	CAG	GAT	48
Met	Lys	Leu	Leu	Glu	Pro	Thr	Asn	Thr	Ser	Tyr	Thr	Leu	Leu	Gln	Asp	
1				5				10					15			
TTA	GCA	TTG	CAT	TTT	GCA	TTT	TAC	TGG	TTT	CTG	GCC	GTG	TAT	ACG	TGG	96
Leu	Ala	Leu	His	Phe	Ala	Phe	Tyr	Trp	Phe	Leu	Ala	Val	Tyr	Thr	Trp	
			20				25						30			
TTA	CCC	GGT	GTC	CTA	GTC	CGG	GCC	GTA	GCT	GTG	GAC	ACA	GGG	GTG	GCT	144
Leu	Pro	Gly	Val	Leu	Val	Arg	Gly	Val	Ala	Val	Asp	Thr	Gly	Val	Ala	
		35				40					45					
CGG	GTG	CCT	GGG	CTC	GGC	CGG	CCC	GGT	AAG	AGG	CTG	CTC	CTG	GCC	GCT	192
Arg	Val	Pro	Gly	Leu	Gly	Arg	Arg	Gly	Lys	Arg	Leu	Leu	Leu	Ala	Ala	
	50					55				60						
GTG	GCT	GTC	TTG	GCG	CTT	GTT	GTG	TCC	GTT	GTT	GTC	CCG	GCT	TAT	GTG	240
Val	Ala	Val	Leu	Ala	Leu	Val	Val	Ser	Val	Val	Val	Pro	Ala	Tyr	Val	
	65			70			75							80		
GCG	TAT	AGT	AGT	CTG	CAC	CCG	GAG	AGC	TGT	CGG	CCC	GTT	GCG	CCG	GAG	288
Ala	Tyr	Ser	Ser	Leu	His	Pro	Glu	Ser	Cys	Arg	Pro	Val	Ala	Pro	Glu	
				85			90						95			
GGG	CTC	ACC	TAC	AAA	GAG	TTC	ACC	GTG	ACC	GCG	GAG	GAT	GGC	TTG	GTG	336
Gly	Leu	Thr	Tyr	Lys	Glu	Phe	Ser	Val	Thr	Ala	Glu	Asp	Gly	Leu	Val	
			100				105					110				
GTT	CGG	GGC	TGG	GTG	CTG	GGC	CCC	GGC	GCT	GGG	GGC	AAC	CCG	GTG	TTC	384
Val	Arg	Gly	Trp	Cal	Leu	Gly	Pro	Gly	Ala	Gly	Gly	Asn	Pro	Val	Phe	
		115				120						125				
GTT	TTG	ATG	CAC	GGG	TAT	ACT	GGG	TGC	CGC	TCG	GCG	CCC	TAC	ATG	GCT	432
Val	Leu	Met	His	Gly	Tyr	Thr	Gly	Cys	Arg	Ser	Ala	Pro	Tyr	Met	Ala	
	130				135					140						
GTG	CTG	GCC	CGG	GAG	CTC	GTG	GAG	TGG	GGG	TAC	CCG	GTG	GTT	GTG	TTC	480
Val	Leu	Ala	Arg	Glu	Leu	Val	Glu	Trp	Gly	Tyr	Pro	Val	Val	Val	Phe	
145					150					155					160	

GAC TTC CGG GGC CAC GGG GAG AGC GGG GGC TCG ACG ACG ATT GGG CCC Asp Phe Arg Gly His Gly Glu Ser Gly Gly Ser Thr Thr Ile Gly Pro 165 170 175	528
CGG GAG GTG CTG GAT GCC CGG GCT GTG GTG GGC TAT GTC TCG GAG CGG Arg Glu Val Leu Asp Ala Arg Ala Val Val Gly Tyr Val Ser Glu Arg 180 185 190	576
TTC CCC GGC CGC CGG ATA ATA TTG GTG GGG TTC AGT ATG GGC GGC GCT Phe Pro Gly Arg Arg Ile Ile Leu Val Gly Phe Ser Met Gly Gly Ala 195 200 205	624
GTA GCG ATC GTG GAG GGT GCT GGG GAC CCG CGG GTC TAC GCG GTG GCT Val Ala Ile Val Glu Gly Ala Gly Asp Pro Arg Val Tyr Ala Val Ala 210 215 220	672
GCT GAT AGC CCG TAC TAT AGG CTC CGG GAC GTC ATA CCC CGG TGG CTG Ala Asp Ser Pro Tyr Tyr Arg Leu Arg Asp Val Ile Pro Arg Trp Leu 225 230 235 240	720
GAG TAC AAG ACG CCG CTG CCG GGC TGG GTG GGT GTG CTG GCC GGG TTC Glu Tyr Lys Thr Leu Pro Gly Trp Val Gly Val Leu Ala Gly Phe 245 250 255	768
TAC GGG AGG CTG ATG GCG GGC GTT GAC CTC GGC TTC GGC CCC GCT GGG Tyr Gly Arg Leu Met Ala Gly Val Asp Leu Gly Phe Gly Pro Ala Gly 260 265 270	816
GTG GAG CGC GTG GAT AAG CCG TTG CTG GTG GTG TAT GGG CCC CGG GAC Val Gly Arg Val Asp Lys Pro Leu Leu Val Val Tyr Gly Pro Arg Asp 275 280 285	864
CCG CTG GTG ACG CGG GAC GAG GCG AGG AGC CTG GCG TCC CGT AGC CCG Pro Leu Val Thr Arg Asp Glu Ala Arg Ser Leu Ala Ser Arg Ser Pro 290 295 300	912
TGT GGC CGT CTC GTC GAG GTT CCT GGG GCT GGC CAC GTG GAG GCC GTG Cys Gly Arg Leu Val Glu Val Pro Gly Ala Gly His Val Glu Ala Val 305 310 315 320	960
GAT GTG CTC GGG CCG GGC CGC TAC GCA GAC ATG CTG ATA GAG CTG GCG Asp Val Leu Gly Pro Gly Arg Tyr Ala Asp Met Leu Ile Glu Leu Ala 325 330 335	1008
CAC GAG GAG TGC CCT CCG GGG GGC GGT GGC TGA His Glu Glu Cys Pro Pro Gly Ala Gly Gly 340 345	1019

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 789 NUCLEOTIDES
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ATG CCA TAT GTT AGG AAT GGT GGT GTA AAT ATC TAT TAT GAA CTG GTG	48
Met Pro Tyr Val Arg Asn Gly Gly Val Ile Tyr Tyr Glu Leu Val	
1 5 10 15	
GAT GGA CCT GAG CCA CCA ATT GTC TTT GTT CAC GGA TGG ACA GCA AAT	96
Asp Gly Pro Glu Pro Pro Ile Val Phe Val His Gly Trp Thr Ala Asn	
20 25 30	
ATG AAT TTT TGG AAA GAG CAA ACA CGT TAT TTT GCA GGC AGG AAT ATG	144
Met Asn Phe Trp Lys Glu Gln Arg Arg Tyr Phe Ala Gly Arg Asn Met	
35 40 45	
ATG TTG TTT GTC GAT AAC AGA GGT CAT GGC AGG TCC GAT AAG CCA CTT	192
Met Leu Phe Val Asp Asn Arg Gly His Gly Arg Ser Asp Lys Pro Leu	
50 55 60	
GGA TAC GAT TTC TAC AGA TTT GAG AAC TTC ATT TCA GAT TTA GAT GCG	240
Gly Tyr Asp Phe Tyr Arg Phe Glu Asn Phe Ile Ser Asp Leu Asp Ala	
65 70 75 80	
GTT GTT AGG GAG ACT GGA GTG GAG AAA TTT GTT CTC GTC GGA CAT TCA	288
Val Val Arg Glu Thr Gly Val Glu Lys Phe Cal Leu Val Gly His Ser	
85 90 95	
TTC GGA ACA ATG ATC TCT ATG AAG TAC TGT TCG GAG TAT CGG AAT CGG	336
Phe Gly Thr Met Ile Ser Met Lys Tyr Cys Ser Glu Tyr Arg Asn Arg	
100 105 110	
GTT CTT GCT CTA ATC CTC ATA GGT GGT GGC AGC AGA ATA AAG CTT CTA	384
Val Leu Ala Leu Ile Leu Ile Gly Gly Ser Arg Ile Lys Leu Leu	
115 120 125	
CAC AGA ATT GGA TAT CCT TTA GCA AAG ATT CTT GCA TCC ATT GCA TAC	432
His Arg Ile Gly Tyr Pro Leu Ala Lys Ile Leu Ala Ser Ile Ala Tyr	
130 135 140	
AAG AAG TCT TCA AGA TTG GTC GCA GAT CTT TCC TTT GGC AAA AAT GCT	480
Lys Lys Ser Ser Arg Leu Val Ala Asp Leu Ser Phe Gly Lys Asn Ala	
145 150 155 160	
GGT GAA CTT AAA GAG TGG GGA TGG AAA CAG GCA ATG GAT TAT ACA CCC	528
Gly Glu Leu Lys Glu Trp Gly Trp Lys Gln Ala Met Asp Tyr Thr Pro	
165 170 175	
TCC TAC GTG GCA ATG GAC ACG TAC AGA ACT CTA ACG AAA GTG AAT CTT	576
Ser Tyr Val Ala Met Tyr Thr Tyr Arg Thr Leu Thr Lys Val Asn Leu	
180 185 190	
GAA AAT ATC TTG GAG AAA ATA GAC TGT CCA ACA CTG ATT ATC GTT GGA	624
Glu Asn Ile Leu Glu Lys Ile Asp Cys Pro Thr Leu Ile Ile Val Gly	
195 200 205	
GAA GAG GAT GCA CTA TTG CCC GTT AGC AAA TCA GTT GAG CTG AGC AGG	672
Glu Glu Asp Ala Leu Leu Pro Val Ser Lys Ser Val Glu Leu Ser Arg	
210 215 220	
AGG ATA GAA AAC TCA AAG CTT GTG ATC ATC CCA AAC TCG GGG CAT TGC	720
Arg Ile Glu Asn Ser Lys Leu Val Ile Ile Pro Asn Ser Gly His Cys	
225 230 235 240	
GTA ATG CTT GAG AGT CCA AGT GAG GTT AAT AGA GCA ATG GAC GAA TTC	768
Val Met Leu Glu Ser Pro Ser Glu Val Asn Arg Ala Met Asp Glu Phe	
245 250 255	

ATT TCT TCA GCA CAG TTC TAA
 Ile Ser Ser Ala Gln Phe
 260

774

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 756 NUCLEOTIDES
- (B) TYPE: NUCLEIC ACID
- (C) STRANDEDNESS: SINGLE
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTG AGA TTG AGG AAA TTT GAA GAG ATA AAC CTC GTT CTT TCG GGA GGA Leu Arg Leu Arg Lys Phe Glu Glu Ile Asn Leu Val Leu Ser Gly Gly 1 5 10 15	48
GCT GCA AAG GGC ATA GCC CAC ATA GGT GTT TTG AAA GCT ATA AAC GAG Ala Ala Lys Gly Ile Ala His Ile Gly Val Leu Lys Ala Ile Asn Glu 20 25 30	96
CTC GGT ATA AGG GTG AGG GCT TTA AGC GGG GTG AGC GCC GGG GCA ATC Leu Glu Ile Arg Val Arg Ala Leu Ser Gly Val Ser Ala Gly Ala Ile 35 40 45	144
GTT TCG GTC TTT TAT GCC TCA GGC TAC TCC CCT GAA GGG ATG TTC AGC Val Ser Val Phe Tyr Ala Ser Gly Tyr Ser Pro Glu Gly Met Phe Ser 50 55 60	192
CTT CTG AAG AGG GTA AAC TGG CTG AAG CTG TTT AAG TTC AAG CCA CCT Leu Leu Lys Arg Val Asn Trp Leu Lys Leu Phe Lys Phe Lye Pro Pro 65 70 75 80	240
CTG AAG GGA TTG ATA GGG TGG GAG AAG GCT ATA AGA TTC CTT GAG GAA Leu Lys Gly Leu Ile Gly Trp Glu Lys Ala Ile Arg Phe Leu Glu Glu 85 90 95	288
GTT CTC CCT TAC AGG AGA ATA GAA AAA CTT GAG ATA CCG ACG TAT ATA Val Leu Pro Tyr Arg Arg Ile Glu Lys Leu Glu Ile Pro Thr Tyr Ile 100 105 110	336
TGC GCG ACG GAT TTA TAC TCG GGA AGG GCT CTA TAC CTC TCG GAA GGG Cys Ala Thr Asp Leu Tyr Ser Gly Arg Ala Leu Tyr Leu Ser Glu Gly 115 120 125	384
AGT TTA ATC CCC GCA CTT CTC GGC AGC TGT GCA ATT CCC GGC ATA TTT Ser Leu Ile Pro Ala Leu Leu Gly Ser Cys Ala Ile Pro Gly Ile Phe 130 135 140	432
GAA CCC GTT GAG TAT AAG AAT TAC TTG CTC GTT GAC GGA GGT ATA GTT Glu Pro Val Glu Tyr Lys Asn Tyr Leu Leu Val Asp Gly Gly Ile Val 145 150 155 160	480
AAC AAC CTT CCC GTT GAG CCC TTT CAG GAA AGC GGT ATT CCC ACC GTT Asn Asn Leu Pro Val Glu Pro Phe Gln Glu Ser Gly Ile Pro Thr Val 165 170 175	528
TGC GTT GAT GTC CTT CCC ATA GAG CCG GAA AAG GAT ATA AAG AAC ATT Cys Val Asp Val Leu Pro Ile Glu Pro Glu Lys Asp Ile Lys Asn Ile 180 185 190	576

CTT CAC ATC CTT TTG AGG AGC TTC TTT CTT GCG GTC CGC TCA AAC TCC	624
Leu His Ile Leu Leu Arg Ser Phe Phe Leu Ala Val Arg Ser Asn Ser	
195 200 205	
GAA AAG AGA AAG GAG TTT TGT GAC CTC GTT ATA GTT CCT GAG CTT GAG	672
Glu Lys Arg Lys Glu Phe Cys Asp Leu Val Ile Val Pro Glu Leu Glu	
210 215 220	
GAG TTC ACA CCC CTT GAT GTT ACA AAA GCG GAC CAA ATA ATG GAG AGG	720
Glu Phe Thr Pro Leu Asp Val Arg Lys Ala Asp Gln Ile Met Glu Arg	
225 230 235 240	
GGA TAC ATA AAG GCC TTA GAG TCA CTT TCT GAA TAG	768
Gly Tyr Ile Lys Ala Leu Glu Val Leu Ser Glu	
245 250	

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 894 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATG TTT AAT ATC AAT GTC TTT GTT AAT ATA TCT TGG CTG TAT TTT TCA	48
Met Phe Asn Ile Asn Val Phe Val Asn Ile Ser Trp Leu Tyr Phe Ser	
1 5 10 15	
GGG ATA GTT ATG AAG ACT GTG GAA GAG TAT GCG CTA CTT GAA ACA GGC	96
Gly Ile Val Met Lys Thr Val Glu Glu Tyr Ala Leu Leu Glu Thr Gly	
20 25 30	
GTA AGA GTG TTT TAT CGG TGT GTA ATC CCG GAG AAA GCT TTT AAC ACT	144
Val Arg Val Phe Tyr Arg Cys Val Ile Pro Glu Lys Ala Phe Asn Thr	
35 40 45	
TTG ATA ATA GGT TCA CAC GGA TTG GGG GCG CAC AGT GGA ATC TAC ATT	192
Leu Ile Ile Gly Ser His Gly Leu Gly Ala His Ser Gly Ile Tyr Ile	
50 55 60	
AGT GTT GCT GAA GAA TTT GCT AGG CAC GGA TTT GGA TTC TGC ATG CAC	240
Ser Val Ala Glu Glu Phe Ala Arg His Gly Phe Gly Phe Cys Met His	
65 70 75 80	
GAT CAA AGG GGA CAT GGG AGA ACG GCA AGC GAT AGA GAA AGA GGG TAT	288
Asp Gln Arg Gly His Gly Arg Thr Ala Ser Asp Arg Glu Arg Gly Tyr	
85 90 95	
GTG GAG GGC TTT CAC AAC TTC ATA GAG GAT ATG AAG GCC TTC TCC GAT	336
Val Glu Gly Phe His Asn Phe Ile Glu Asp Met Lys Ala Phe Ser Asp	
100 105 110	
TAT GCC AAG TGG CGC GTG GGA GGT GAC GAA ATA ATA TTG CTA GGA CAC	384
Tyr Ala Lys Trp Arg Val Gly Gly Asp Glu Ile Ile Leu Leu Gly His	
115 120 125	
AGT ATG GGC GGG CTG ATA GCG CAC GGA ACA GTT GCA ACT TAT AAA GAA	432
Ser Met Gly Gly Leu Ile Ala Leu Leu Thr Val Ala Thr Tyr Lys Glu	

130	135	140	
ATC GCC AAG GGA GTT ATC GCG CTA GCC CCG GCC CTC CAA ATC CCC TTA Ile Ala Lys Gly Val Ile Ala Leu Ala Pro Ala Leu Gln Ile Pro Leu 145 150 155 160			480
ACC CCG GCT AGA AGA CTT GTT CTA AGC CTC GCG TCA AGG CTT GCC CCG Thr Pro Ala Arg Arg Leu Val Leu Ser Leu Ala Ser Arg Leu Ala Pro 165 170 175			528
CAT TCT AAG ATC ACC TTA CAA ACG AGA TTG CCG CAG AAA CCA GAG GGT His Ser Lys Ile Thr Leu Gln Arg Arg Leu Pro Gln Lys Pro Glu Gly 180 185 190			576
TTT CAA AGA GCA AAA GAT ATA GAA TAC AGT CTG AGT GAA ATA TCA GTC Phe Gln Arg Ala Lys Asp Ile Glu Tyr Ser Leu Ser Glu Ile Ser Val 195 200 205			624
AAG CTC GTG GAC GAA ATG ATT AAA GCA TCA TCT ATG TCT TGG ACC ATA Lys Leu Val Asp Glu Met Ile Lys Ala Ser Ser Met Phe Trp Thr Ile 210 215 220			672
GCA GGG GAA ATT AAT ACT CCC GTC CTG CTT ATT CAT GGG GAA AAA CAG Ala Gly Glu Ile Asn Thr Pro Val Leu Leu Ile His Gly Glu Lys Asp 225 230 235 240			720
AAT GTC ATA CCT CCG GAG GCG ACC AAA AAA GCC RTAC CAA TTA ATA CCT Asn Val Ile Pro Pro Glu Ala Ser Lys Lys Als Tyr Gln Leu Ile Pro 245 250 255			768
TCA TTC CCT AAA GAG TTG AAA AAA TAC CCC GAT CTT GGA CAC AAC TTG Ser Phe Pro Lys Glu Leu Lys Ile Tyr Pro Asp Leu Gly His Asn Leu 260 265 270			816
TTT TTT GAA CCA GGC GCG GTG AAA ATC GTC ACA GAC ATT GTA GAG TGG Phe Phe Glu Pro Gly Ala Val Lys Ile Val Thr Asp Ile Val Glu Trp 275 280 285			864
GTT AAG AAT CTA CCC AGG GAA AAT CCT TAA Val Lys Asn Leu Pro Arg Glu Asn Pro 290 295			874

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 789 NUCLEOTIDES
 - (B) TYPE: NUCLEIC ACID
 - (C) STRANDEDNESS: SINGLE
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATG GAG GTT TAC AAG GCC AAA TTC GGC GAA GCA AAG CTC GGC TGG GTC Met Glu Val Tyr Lys Ala Lys Phe Gly Glu Ala Lys Leu Gly Trp Val 1 5 10 15	48
GTT CTG GTT CAT GGC CTC GGC GAG CAC AGC GGA AGG TAT GGA AGA CTG Val Leu Val His Gly Leu Gly Glu His Ser Gly Arg Tyr Gly Arg Leu 20 25 30	96
ATT AAG GAA CTC AAC TAT GCC GGC TTT GGA GTT TAC ACC TTC GAC TGG Ile Lys Glu Leu Asn Tyr Ala Gly Phe Gly Val Tyr Thr Phe Asp Trp	144

35	40	45	
CCC GGC CAC GGG AAG AGC CCG GGC AAG AGA GGG CAC ACG AGC GTC GAG Pro Gly His Gly Lys Ser Pro Gly Lys Arg Gly His Thr Ser Val Glu 50 55 60			192
GAG GCG ATG GAA ATC ATC GAC TCG ATA ATC GAG GAG ATC AGG GAG AAG Glu Ala Met Glu Ile Ile Asp Ser Ile Ile Glu Glu Ile Arg Glu Lys 65 70 75 80			240
CCC TTC CTC TTC GGC CAC AGC CTC GGT GGT CTA ACT GTC ATC AGG TAC Pro Phe Leu Phe Gly His Ser Leu Gly Gly Leu Thr Val Ile Arg Tyr 85 90 95			288
GCT GAG ACG CGG CCC GAT AAA ATA CGG GGA TTA ATA GCT TCC TCG CCT Ala Glu Thr Arg Pro Asp Lys Ile Arg Gly Leu Ile Ala Ser Ser Pro 100 105 110			336
GCC CTC GCC AAG AGC CCG GAA ACG CCG GGC TTC ATG GTG GCC CTC GCG Ala Leu Ala Lys Ser Pro Glu Thr Pro Gly Phe Met Val Ala Leu Ala 115 120 125			384
AAG TTC CTT GGA AAG ATC GCC CCG GGA GTT GTT CTC TCC AAC GGC ATA Lys Phe Leu Gly Lys Ile Ala Pro Gly Val Val Leu Ser Asn Gly Ile 130 135 140			432
AAG CCG GAA CTC CTC TCG AGG AAC AGG GAC GCC GTG AGG AGG TAC GTT Lys Pro Glu Leu Leu Ser Arg Asn Arg Asp Ala Val Arg Arg Tyr Val 145 150 155 160			480
GAA GAC CCA CTC GRC CAC GAC AGG ATT TCG GCC AAG CTG GGA AGG AGC Glu Asp Pro Leu Val His Asp Arg Ile Ser Ala Lys Leu Gly Arg Ser 165 170 175			528
ATC TTC GTG AAC ATG GAG CTG GCC CAC AGG GAG GCG GAC AAG ATA AAA Ile Phe Val Asn Met Glu Leu Ala His Arg Glu Ala Asp Lys Ile Lys 180 185 190			576
GTC CCG ATC CTC CTT CTG ATC GCC ACT GGC GAT GTA ATA ACC CCG CCT Val Pro Ile Leu Leu Leu Ile Gly Thr Gly Asp Val Ile Thr Pro Pro 195 200 205			624
GAA GGC TCA CGC AGA CTC TTC GAG GAG CTG GCC GTC GAG AAC AAA ACC Glu Gly Ser Arg Arg Leu Phe Glu Glu Leu Ala Val Glu Asn Lys Thr 210 215 220			672
CTG AGG GAG TTC GAG GGG GCG TAC CAC GAG ATA TTT GAA GAC CCC GAG Leu Arg Glu Phe Glu Gly Ala Tyr His Glu Ile Phe Glu Asp Pro Glu 225 230 235 240			720
TGG GCC GAG GAG TTC CAC GAA ACA ATT GTT AAG TGG CTG GTT GAA AAA Trp Ala Glu Glu Phe His Glu Thr Ile Val Lys Trp Leu Val Glu Lys 245 250 255			768
TCG TAC TCT TCG GCT CAA TAA Ser Tyr Ser Ser Ala Gln 260			775

- (2) INFORMATION FOR SEQ ID NO:29:
 (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 750 NUCLEOTIDES
 (B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAF

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTG ATT GGC AAT TTG AAA TTG AAG AGG TTT GAA GAG GTT AAC TTA GTT Leu Ile Gly Asn Leu Lys Ley Lys Arg Phe Glu Glu Val Asn Leu Val 1 5 10 15	48
CTT TCG GGA GGG GCT GCC AAG GCT ATC GCC CAT ATA GGT GTT TTA AAA Leu Ser Gly Gly Ala Ala Lys Gly Ile Ala His Ile Gly Val Leu Lys 20 25 30	96
GCT CTG GAA GAG CTC GGT ATA AAG GTA AAG AGG CTC AGC GGG GTA AGT Ala Leu Glu Glu Leu Gly Ile Lys Val Lys Arg Leu Ser Gly Val Ser 35 40 45	144
GCT GGA GCT ATC GTT TCC GTC TTT TAC GCT TCG GGC TAC ACT CCC GAC Ala Gly Ala Ile Val Ser Val Phe Tyr Ala Ser Gly Tyr Thr Pro Asp 50 55 60	192
GAG ATG TTA AAA CTC CTG AAA GAG GTA AAC TGG CTC AAA CTT TTT AAG Glu Met Leu Lys Leu Leu Lys Glu Val Asn Trp Leu Lys Leu Phe Lys 65 70 75 80	240
TTC AAA ACA CCG AAA ATG GGC TTA ATG GGG TGG GAG AAG GCT GCA GAG Phe Lys Thr Pro Lys Met Gly Leu Met Gly Trp Glu Lys Ala Ala Glu 85 90 95	288
TTT TTG TAA AAA GAG CTC GGA GTT AAG AGG CTG GAA GAC CTG AAC ATA Phe Leu Glu Lys Glu Leu Gly Val Lys Arg Leu Glu Asp Leu Asn Ile 100 105 110	336
CCA ACC TAT CTT TGC TCG GCG GAT CTG TAC ACG GGA AAG GCT CTT TAC Pro Thr Tyr Leu Cys Ser Ala Asp Ley Tyr Thr Gly Lys Ala Leu Tyr 115 120 125	384
TTC GGC AGA GGT GAC TTA ATT CCC GTG CTT CTC GGA AGT TGT TCC ATA Phe Gly Arg Gly Asp Leu Ile Pro Val Leu Leu Gly Ser Lys Ser Ile 130 135 140	432
CCC GGG ATT TTT GAA CCA GTT GAG TAC GAG AAT TTT CTA CTT GTT GAC Pro Gly Ile Phe Glu Pro Val Glu Tyr Glu Asn Phe Leu Leu Val Asp 145 150 155 160	480
GGA GGT ATA GTG AAC AAC CTG CCC GTA GAA CCT TTG GAA AAG TTC AAA Gly Gly Ile Val Asn Asn Leu Pro Val Glu Pro Leu Glu Lys Phe Lys 165 170 175	528
GAA CCC ATA ATC GGG GTA GAT GTG CTT CCC ATA ACT CAA GAA AGA AAG Glu Pro Ile Ile Gly Val Asp Val Leu Pro Ile Thr Gln Glu Arg Lys 180 185 190	576
ATT AAA AAT ATA CTC CAC ATC CTT ATA AGG AGC TTC TTT CTG GCG GTT Ile Lys Asn Ile Leu His Ile Leu Ile Arg Ser Phe Phe Leu Ala Val 195 200 205	624
CGT TCC AAT TCG GAA AAG AGA AAG GAG TTC TGC AAC GTA GTT ATA GAA Arg Ser Asn Ser Glu Lys Arg Lys Glu Phe Cys Asn Val Val Ile Glu 210 215 220	672
CCT CCC CTT GAA GAG TTC TCT CCT CTG GAC GTA AAT AAG GCG GAC GAG Pro Pro Leu Glu Glu Phe Ser Pro Leu Asp Val Asn Lys Ala Asp Glu	720

225

230

235

240

ATA TTC TGC GGG GAT ATG AGA GCA CTT TAA
 Ile Phe Cys Gly Asp Met Arg Ala Leu
 245

730

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1017 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATG CCA GCT AAT GAC TCA CCC ACG ATC GAC TTT AAT CCT CGC GGC ATT	48
Met Pro Ala Asn Asp Ser Pro Thr Ile Asp Phe Asn Pro Arg Gly Ile	
1 5 10 15	
CTT CGC AAC GCT CAC GCA CAG GTT ATT TTA GCG ACT TCC GGC TTG CGC	96
Leu Arg Asn Ala His Ala Gln Val Ile Leu Ala Thr Ser Gly Leu Arg	
20 25 30	
AAA GCG TTT TTG AAA CGC ACG CAC AAG AGC TAC CTC AGC ACT GCC CAA	144
Lys Ala Phe Leu Lys Arg Thr His Lys Ser Tyr Leu Ser Thr Ala Gln	
35 40 45	
TGG CTG GAG CTC GAT GCC GGC AAC GGA GTT ACC TTG GCC GGA GAG CTT	192
Trp Leu Glu Leu Asp Ala Gly Asn Gly Val Thr Leu Ala Gly Glu Leu	
50 55 60	
AAC ACA GCG CCT GCA ACT GCA TCC TCC TCC CAC CCG GCG CAC AAG AAC 240	
Asn Thr Ala Pro Ala Thr Ala Ser Ser Ser His Pro Ala His Lys Asn	
65 70 75 80	
ACT CTG GTT ATT GTG CTG CAC GGC TGG GAA GGC TCC AGC CAG TCG GCC	288
Thr Leu Val Ile Val Leu His Gly Trp Glu Gly Ser Ser Gln Ser Ala	
85 90 95	
TAT GCG ACC TCC GCT GGC AGC ACG CTT TTC GAC AAT GGG TTC GAC ACT	336
Tyr Ala Thr Ser Ala Gly Ser Thr Leu Phe Asp Asn Gly Phe Asp Thr	
100 105 110	
TTT CGC CTT AAT TTT CGC GAT CAC GGC GAC ACC TAC CAC TTA AAC CGC	384
Phe Arg Leu Asn Phe Arg Asp His Gly Asp Thr Tyr His Leu Asn Arg	
115 120 125	
GGC ATA TTT AAC TCA TCG CTG ATT GAC GAA GTA GTG GGC GCA GTC AAA	432
Gly Ile Phe Asn Ser Ser Leu Ile Asp Glu Val Val Gly Ala Val Lys	
130 135 140	
GCC ATC CAG CAG CAA ACC GAC TAC GAC AAG TAT TGC CTG ATG GGG TTC	480
Ala Ile Gln Gln Gln Thr Asp Tyr Asp Lys Tyr Cys Leu Met Gly Phe	
145 150 155 160	
TCA CTG GGT GGG AAC TTT GCC TTG CGC GTC GCG GTG CGG GAA CAG CAT	528
Ser Leu Gly Gly Asn Phe Ala Leu Arg Val Ala Val Arg Glu Gln His	
165 170 175	

CTC GCT AAA CCG CTA GCG GGC GTG CTC GCC GTA TGC CCG GTA CTC GAC Leu Ala Lys Pro Leu Ala Gly Val Leu Ala Val Cys Pro Val Leu Asp 180 185 190	576
CCC GCA CAC ACC ATG ATG GCC CTA AAC CGA GGT GCG TTT TTC TAC GGC Pro Ala His Thr Met Met Ala Leu Asn Arg Gly Ala Phe Phe Tyr Gly 195 200 205	624
CGC TAT TTT GCG CAT AAA TGG AAG CGC TCG TTA ACC GCA AAA CTT GCA Arg Tyr Phe Ala His Lys Trp Lys Arg Ser Leu Thr Ala Lys Leu Ala 210 215 220 225	672
GCT TTC CCA GAC TAC AAA TAC GGC AAA GAT TTA AAA TCG ATA CAC ACG Ala Phe Pro Asp Tyr Lys Tyr Gly Lys Asp Leu Lys Ser Ile His Thr 230 235 240	720
CTT GAT GAG TTA AAC AAC TAT TTC ATT CCC CGC TAC ACC GGC TTC AAC Leu Asp Glu Leu Asn Asn Tyr Phe Ile Pro Arg Tyr Thr Gly Phe Asn 245 250 255	768
TCA GTC TCC GAA TAC TTC AAA AGT TAC ACG CTC ACC GGC CAG AAG CTC Ser Val Ser Glu Tyr Phe Lys Ser Tyr Thr Leu Thr Gly Gln Lys Leu 260 265 270	816
GCG TTT CTC AAC TGC CCC AGT TAC ATT CTG GCA GCT GGC GAC GAC CCA Ala Phe Leu Asn Cys Pro Ser Tyr Ile Leu Ala Ala Gly Asp Asp Pro 275 280 285	864
ATA ATT CCA GCA TCC GAC TTT CAG AAA ATA GCC AAG CCT GCG AAT CTG Ile Ile Pro Ala Ser Asp Phe Gln Lys Ile Ala Lys Pro Ala Asn Leu 290 295 300 305	912
CAC ATA ACA GTA ACG CAA CAA GGT TCT CAT TGC GCA TAC CTG GAA AAC His Ile Thr Val Thr Gln Gln Gly Ser His Cys Ala Tyr Leu Glu Asn 310 315 320	960
CTG CAT AAA CCT AGT GCT GCC GAC AAA TAT GCG GTG AAA TTA TTT GGA Leu His Lys Pro Ser Ala Ala Asp Lys Tyr Ala Val Lys Leu Phe Gly 325 330 335	1,008
GCC TGT TGA Ala Cys	1,111

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 936 NUCLEOTIDES
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

ATG CTT GAT ATG CCA ATC GAC CCT GTT TAC TAC CAG CTT GCT GAG TAT Met Leu Asp Met Pro Ile Asp Pro Val Tyr Tyr Gln Leu Ala Glu Tyr 1 5 10 15	48
TTC GAC AGT CTG CCG AAG TTC GAC CAG TTT TCC TCG GCC AGA GAG TAC Phe Asp Ser Leu Pro Lys Phe Asp Gln Phe Ser Ser Ala Arg Glu Tyr 20 25 30	96
AGG GAG GCG ATA AAT CGA ATA TAC GAG GAG AGA AAC CGG CAG CTG AGC	144

Arg	Glu	Ala	Ile	Asn	Arg	Ile	Tyr	Glu	Glu	Arg	Asn	Arg	Gln	Leu	Ser		
		35					40					45					
CAG	CAT	GAG	AGG	GTT	GAA	AGA	GTT	GAG	GAC	AGG	ACG	ATT	AAG	GGG	AGG		192
Gln	His	Glu	Arg	Val	Glu	Arg	Val	Glu	Asp	Arg	Thr	Ile	Lys	Gly	Arg		
		50				55					60						
AAC	GGA	GAC	ATC	AGA	GTC	AGA	GTT	TAC	CAG	CAG	AAG	CCC	GAT	TCC	CCG		240
Asn	Gly	Asp	Ile	Arg	Val	Arg	Val	Tyr	Gln	Gln	Lys	Pro	Asp	Ser	Pro		
		65			70					75					80		
GGT	CTG	GTT	TAC	TAT	CAC	GGT	GGT	GGA	TTT	GTG	ATT	TGC	AGC	ATC	GAG		288
Val	Leu	Val	Tyr	Tyr	His	Gly	Gly	Gly	Phe	Val	Ile	Cys	Ser	Ile	Glu		
				85				90						95			
TCG	CAC	GAC	GCC	TTA	TGC	AGG	AGA	AYY	GCG	AGA	CTT	TCA	AAC	TCT	ACC		336
Ser	His	Asp	Ala	Leu	Cys	Arg	Arg	Ile	Ala	Arg	Leu	Ser	Asn	Ser	Thr		
			100					105					110				
GTA	GTC	TCC	GTG	GAT	TAC	AGG	CTC	GCT	CCT	GAG	CAC	AAG	TTT	CCC	CCC		384
Val	Val	Ser	Val	Asp	Tyr	Arg	Leu	Ala	Pro	Glu	His	Lys	Phe	Pro	Ala		
		115					120					125					
CCA	GTT	TAT	CAT	TGC	TAC	GAT	GCG	ACC	AAG	TGG	GTT	GCT	GAG	AAC	CGG		432
Ala	Val	Tyr	Asp	Cys	Tyr	Asp	Ala	Thr	Lys	Trp	Val	Ala	Glu	Asn	Ala		
		130				135					140						
GAG	GAG	CTG	AGG	ATT	GAC	CCG	TCA	AAA	ATC	TTC	GTT	GGG	GGG	GAC	AGT		480
Glu	Glu	Leu	Arg	Ile	Asp	Pro	Ser	Lys	Ile	Phe	Val	Gly	Gly	Asp	Ser		
		145			150					155					160		
GCG	GGA	CGG	AAT	CTT	GCC	CCG	GCG	CTT	TCA	ATA	ATG	GCG	AGA	GAC	AGC		528
Ala	Gly	Gly	Asn	Leu	Ala	Ala	Ala	Val	Ser	Ile	Met	Ala	Arg	Asp	Ser		
				165					170					175			
GGA	GAA	GAT	TTC	ATA	AAG	CAT	CAA	ATT	CTA	ACT	TAC	CCC	GTT	GTG	AAC		576
Gly	Glu	Asp	Phe	Ile	Lys	His	Gln	Ile	Leu	Ile	Tyr	Pro	Val	Val	Asn		
			180					185					190				
TTT	GTA	GCC	CCC	ACA	CCA	TCG	CTT	CTG	GAG	TTT	GGA	GAG	GGG	CTG	TGG		624
Phe	Val	Ala	Pro	Thr	Pro	Ser	Leu	Leu	Glu	Phe	Gly	Glu	Gly	Leu	Trp		
		195					200					205					
ATT	CTC	GAC	CAG	AAG	ATA	ATG	AGT	TGG	TTC	TCG	GAG	CAG	TAC	TTC	TCC		672
Ile	Leu	Asp	Gln	Lys	Ile	Met	Ser	Trp	Phe	Ser	Glu	Gln	Tyr	Phe	Ser		
		210				215					230						
AGA	GAG	GAA	GAT	AAG	TTC	AAG	CCC	CTC	GCC	TCC	GTA	ATC	TTT	GCG	GAC		720
Arg	Glu	Glu	Asp	Lys	Phe	Asn	Pro	Leu	Ala	Ser	Val	Ile	Phe	Ala	Asp		
		235			240					245					250		
CTT	GAG	AAC	CTA	CCT	CCT	GCG	CTG	ATC	ATA	ACC	GCC	GAA	TAC	GAC	CCG		768
Leu	Glu	Asn	Leu	Pro	Pro	Ala	Leu	Ile	Ile	Thr	Ala	Glu	Tyr	Asp	Pro		
				255					260					265			
CTG	AGA	GAT	GAA	GGA	GAA	GTT	TTC	GGG	CAG	ATG	CTG	AGA	AGA	GCC	GGT		816
Leu	Arg	Asp	Glu	Gly	Glu	Val	Phe	Gly	Gln	Met	Leu	Arg	Arg	Ala	Gly		
			270					275					280				
GTT	GAG	GCG	AGC	ATC	GTC	AGA	TAC	AGA	GGC	GTG	CTT	CAC	GGA	TTC	ATC		864
Val	Glu	Ala	Ser	Ile	Val	Arg	Tyr	Arg	Gly	Val	Leu	His	Gly	Phe	Ile		
		285					290					295					
AAT	TAC	TAT	CCC	GTG	CTG	AAG	GCT	GCG	AGG	GAT	GCG	ATA	AAC	CAG	ATT		912
Asn	Tyr	Tyr	Pro	Val	Leu	Lys	Ala	Ala	Arg	Asp	Ala	Ile	Asn	Gln	Ile		

300	305	310	
GCC GCT CTT CTT GTG TTC GAC TAG			936
Ala Ala Leu leu Val Phe Asp			
315	320		

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 918 NUCLEOTIDES
 (B) TYPE: NUCLEIC ACID
 (C) STRANDEDNESS: SINGLE
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: GENOMIC DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ATG CCC CTA GAT CCT AGA ATT AAA AAG TTA CTA GAA TCA GCT CTT ACT	48
Met Pro Leu Asp Pro Arg Ile Lys Lys Leu Leu Glu Ser Ala Leu Thr	
5 10 15	
ATA CCA ATT GGT AAA GCC CCA GTA GAA GAG GTA AGA AAG ATA TTT AGG	96
Ile Pro Ile Gly Lys Ala Pro Val Glu Glu Val Arg Lys Ile Phe Arg	
20 25 30	
CAA TTA GCG TCG GCA GCT CCC AAA GTC GAA GTT GGA AAA GTA GAA GAT	144
Gln Leu Ala Ser Ala Ala Pro Lys Val Glu Val Gly Lys Val Glu Asp	
35 40 45	
ATA AAA ATA CCA GGC AGT GAA ACC GTT ATA AAC GCT AGA GTG TAT TTT	192
Ile Lys Ile Pro Gly Ser Glu Thr Val Ile Asn Ala Arg Val Tyr Phe	
50 55 60	
CCG AAG AGT AGC GGT CCT TAT GGT GTT CTA GTG TAT CTT CAT GGA GGC	240
Pro Lys Ser Ser Gly Pro Tyr Gly Val Leu Val Tyr Leu His Gly Gly	
65 70 75 80	
GGT TTT GTA ATA GGC GAT GTG GAA TCT TAT GAC CCA TTA TGT AGA GCA	288
Gly Phe Val Ile Gly Asp Val Glu Ser Tyr Asp Pro Leu Cys Arg Ala	
85 90 95	
ATT ACA AAT GCG TGC AAT TGC GTT GTA GTA TCA GTG GAC TAT AGG TTA	336
Ile Thr Asn Ala Cys Asn Cys Val Val Ser Val Asp Tyr Arg Leu	
100 105 110	
GCT CCA GAA TAC AAG TTT CCT TCT GCA GTT ATC GAT TCA TTT GAC GCT	384
Ala Pro Glu Tyr Lys Phe Pro Ser Ala Val Ile Asp Ser Phe Asp Ala	
115 120 125	
ACT AAT TGG GTT TAT AAC AAT TTA GAT AAA TTT GAT GGA AAG ATG GGA	432
Thr Asn Trp Val Tyr Asn Asn Leu Asp Lys Phe Asp Gly Lys Met Gly	
130 135 140	
GTT GCG ATT GCG GGA GAT AGT GCT GGA GGA AAT TTG GCA GCG GTT GTA	480
Val Ala Ile Ala Gly Asp Ser Ale Gly Gly Asn Leu Ala Ala Val Val	
145 150 155 160	
GCT CTT CTT TCA AAG GGT AAA AAT AAT TTG AAG TAT CAA ATA CTG GTT	528
Ala Leu Leu Ser Lys Gly Lys Ile Asn Leu Lys Tyr Gln Ile Leu Val	
165 170 175	
TAC CCA GCG GTA AGT TTA GAT AAC GTT TCA AGA TCC ATG ATA GAG TAC	576

Tyr	Pro	Ala	Val	Ser	Leu	Asp	Asn	Val	Ser	Arg	Ser	Met	Ile	Glu	Tyr		
			180					185					190				
TCT	GAT	GGG	TTC	TTC	CTT	ACC	ACA	GAG	CAT	ATA	GAG	TGG	TTC	GGT	TCT		624
Ser	Asp	Gly	Phe	Phe	Leu	Thr	Arg	Glu	His	Ile	Glu	Trp	Phe	Gly	Ser		
		195					200				205						
CAA	TAC	TTA	CGA	AGC	CCT	GCA	GAT	TTG	CTA	GAC	TTT	AGG	TTC	TCT	CCA		672
Gln	Tyr	Leu	Arg	Ser	Pro	Ala	Asp	Leu	Leu	Asp	Phe	Arg	Phe	Ser	Pro		
	210					215				220							
ATT	CTG	GCG	CAA	GAT	TTC	AAC	GCA	TTA	CCT	CCA	GCC	TTG	ATA	ATA	ACA		720
Ile	Leu	Ala	Gln	Asp	Phe	Asn	Gly	Leu	Pro	Pro	Ala	Leu	Ile	Ile	Thr		
	225				230				235						240		
GCA	GAA	TAC	GAT	CCA	CTA	AGG	GAT	CAA	GGA	GAA	GCG	TAT	GCA	AAT	AAA		768
Ala	Glu	Tyr	Asp	Pro	Leu	Arg	Asp	Gln	Gly	Glu	Ala	Tyr	Ala	Asn	Lys		
				245				250					255				
CTA	CTA	CAA	GCT	GGA	GTC	TCA	GTT	ACT	AGT	GTG	AGA	TTT	AAC	AAC	GTT		816
Leu	Leu	Gln	Ala	Gly	Val	Ser	Val	Thr	Ser	Val	Arg	Phe	Asn	Asn	Val		
		260					265						270				
ATA	CAC	GGA	TTC	CTC	TCA	TTC	TTT	CCG	TTG	ATG	GAG	CAA	GGA	AGA	GAT		864
Ile	His	Gly	Phe	Leu	Ser	Phe	Phe	Pro	Leu	Met	Glu	Gln	Gly	Arg	Asp		
		275					280					285					
GCT	ATA	GGT	CTG	ATA	GGG	TCT	GTG	TTA	AGA	CGA	GTA	TTT	TAT	GAT	AAA		912
Ala	Ile	Gly	Leu	Ile	Gly	Ser	Val	Leu	Arg	Arg	Val	Phe	Tyr	Asp	Lys		
	290					295					300						
ATT	TAA																918
Ile																	
305																	

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 184 AMINO ACIDS
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met	Ser	Leu	Asn	Lys	His	Ser	Trp	Met	Asp	Met	Ile	Ile	Phe	Ile	Leu		
1				5				10					15				
Ser	Phe	Ser	Phe	Pro	Leu	Thr	Met	Ile	Ala	Leu	Ala	Ile	Ser	Met	Ser		
		20					25					30					
Ser	Trp	Phe	Asn	Ile	Trp	Asn	Asn	Ala	Leu	Ser	Asp	Leu	Gly	His	Ala		
	35					40					45						
Val	Lys	Ser	Ser	Val	Ala	Pro	Ile	Phe	Asn	Leu	Gly	Leu	Ala	Ile	Gly		
	50				55						60						
Gly	Ile	Leu	Ile	Val	Ile	Val	Gly	Leu	Arg	Asn	Leu	Tyr	Ser	Trp	Ser		
	65				70				75					80			
Arg	Val	Lys	Gly	Ser	Leu	Ile	Ile	Ser	Met	Gly	Val	Phe	Leu	Asn	Leu		
			85					90						95			

Ile Gly Val Phe Asp Glu Val Tyr Gly Trp Ile His Phe Leu Val Ser
100 105 110
Val Leu Phe Phe Leu Ser Ile Ile Ala Tyr Phe Ile Ala Ile Ser Ile
115 120 125
Leu Asp Lys Ser Trp Ile Ala Val Leu Leu Ile Ile Gly His Ile Ala
130 135 140
Met Trp Tyr Leu His Phe Ala Ser Glu Ile Pro Arg Gly Ala Ala Ile
145 150 155 160
Pro Glu Leu Leu Ala Val Phe Ser Phe Leu Pro Phe Tyr Ile Arg Asp
165 170 175
Tyr Phe Lys Ser Tyr Thr Lys Arg
180

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 346 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Met Lys Leu Leu Glu Pro Thr Asn Thr Ser Tyr Thr Leu Leu Gln Asp
1 5 10 15
Leu Ala Leu His Phe Ala Phe Tyr Trp Phe Leu Ala Val TYR Thr Trp
20 25 30
Leu Pro Gly Val Leu Val Arg Gly Val Ala Val Asp Thr Gly Val Ala
35 40 45
Arg Val Pro Gly Leu Gly Arg Arg Gly Lys Arg Leu Leu Leu Ala Ala
50 55 60
Val Ala Val Leu Ala Leu Val Val Ser Val Val Val Pro Ala Tyr Val
65 70 75 80
Ala Tyr Ser Ser Leu His Pro Glu Ser Cys Arg Pro Val Ala Pro Glu
85 90 95
Gly Leu Thr Tyr Lys Glu Phe Ser Val Thr Ala Glu Asp Gly Leu Val
100 105 110
Val Arg Gly Trp Cal Leu Gly Pro Gly Ala Gly Gly Asn Pro Val Phe
115 120 125
Val Leu Met His Gly Tyr Thr Gly Cys Arg Ser Ala Pro Tyr Met Ala
130 135 140
Val Leu Ala Arg Glu Leu Val Glu Trp Gly Tyr Pro Val Val Val Phe
145 150 155 160
Asp Phe Arg Gly His Gly Glu Ser Gly Gly Ser Thr Thr Ile Gly Pro
165 170 175
Arg Glu Val Leu Asp Ala Arg Ala Val Val Gly Tyr Val Ser Glu Arg
180 185 190

Phe Pro Gly Arg Arg Ile Ile Leu Val Gly Phe Ser Met Gly Gly Ala
 195 200 205
 Val Ala Ile Val Glu Gly Ala Gly Asp Pro Arg Val Tyr Ala Val Ala
 210 215 220
 Ala Asp Ser Pro Tyr Tyr Arg Leu Arg Asp Val Ile Pro Arg Trp Leu
 225 230 235 240
 Glu Tyr Lys Thr Pro Leu Pro Gly Trp Val Gly Val Leu Ala Gly Phe
 245 250 255
 Tyr Gly Arg Leu Met Ala Gly Val Asp Leu Gly Phe Gly Pro Ala Gly
 260 265 270
 Val Gly Arg Val Asp Lys Pro Leu Leu Val Val Tyr Gly Pro Arg Asp
 275 280 285
 Pro Leu Val Thr Arg Asp Glu Ala Arg Ser Leu Ala Ser Arg Ser Pro
 290 295 300
 Cys Gly Arg Leu Val Glu Val Pro Gly Ala Gly His Val Glu Ala Val
 305 310 315 320
 Asp Val Leu Gly Pro Gly Arg Tyr Ala Asp Met Leu Ile Glu Leu Ala
 325 330 335
 His Glu Glu Cys Pro Pro Gly Ala Gly Gly
 340 345

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 262 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met Pro Tyr Val Arg Asn Gly Gly Val Asn Ile Tyr Tyr Glu Leu Val
 1 5 10 15
 Asp Gly Pro Glu Pro Pro Ile Val Phe Val His Gly Trp Thr Ala Asn
 20 25 30
 Met Asn Phe Trp Lys Glu Gln Arg Arg Tyr Phe Ala Gly Arg Asn Met
 35 40 45
 Met Leu Phe Val Asp Asn Arg Gly His Gly Arg Ser Asp Lys Pro Leu
 50 55 60
 Gly Tyr Asp Phe Tyr Arg Phe Glu Asn Phe Ile Ser Asp Leu Asp Ala
 65 70 75 80
 Val Val Arg Glu Thr Gly Val Glu Lys Phe Cal Leu Val Gly His Ser
 85 90 95
 Phe Gly Thr Met Ile Ser Met Lys Tyr Cys Ser Glu Tyr Arg Asn Arg
 100 105 110
 Val Leu Ala Leu Ile Leu Ile Gly Gly Gly Ser Arg Ile Lys Leu Leu

115	120	125
His Arg Ile Gly Tyr Pro	Leu Ala Lys Ile Leu	Ala Ser Ile Ala Tyr
130	135	140
Lys Lys Ser Ser Arg Leu	Val Ala Asp Leu Ser	Phe Gly Lys Asn Ala
145	150	155
Gly Glu Leu Lys Glu Trp	Gly Trp Lys Gln Ala	Met Asp Tyr Thr Pro
165	170	175
Ser Tyr Val Ala Met Tyr	Thr Tyr Arg Thr Leu	Thr Lys Val Asn Leu
180	185	190
Glu Asn Ile Leu Glu Lys	Ile Asp Cys Pro Thr	Leu Ile Ile Val Gly
195	200	205
Glu Glu Asp Ala Leu Leu	Pro Val Ser Lys Ser	Val Glu Leu Ser Arg
210	215	220
Arg Ile Glu Asn Ser Lys	Leu Val Ile Ile Pro	Asn Ser Gly His Cys
225	230	235
Val Met Leu Glu Ser Pro	Ser Glu Val Asn Arg	Ala Met Asp Glu Phe
245	250	255
Ile Ser Ser Ala Gln Phe		
260		

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 251 AMINO ACIDS
- (B) TYPE: AMINO ACID
- (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Leu Arg Leu Arg Lys Phe Glu Glu Ile Asn Leu Val Leu Ser Gly Gly
1 5 10 15
Ala Ala Lys Gly Ile Ala His Ile Gly Val Leu Lys Ala Ile Asn Glu
20 25 30
Leu Glu Ile Arg Val Arg Ala Leu Ser Gly Val Ser Ala Gly Ala Ile
35 40 45
Val Ser Val Phe Tyr Ala Ser Gly Tyr Ser Pro Glu Gly Met Phe Ser
50 55 60
Leu Leu Lys Arg Val Asn Trp Leu Lys Leu Phe Lys Phe Lys Pro Pro
65 70 75 80
Leu Lys Gly Leu Ile Gly Trp Glu Lys Ala Ile Arg Phe Leu Glu Glu
85 90 95
Val Leu Pro Tyr Arg Arg Ile Glu Lys Leu Glu Ile Pro Thr Tyr Ile
100 105 110
Cys Ala Thr Asp Leu Tyr Ser Gly Arg Ala Leu Tyr Leu Ser Glu Gly
115 120 125

Ser Leu Ile Pro Ala Leu Leu Gly Ser Cys Ala Ile Pro Gly Ile Phe
130 135 140

Glu Pro Val Glu Tyr Lys Asn Tyr Leu Leu Val Asp Gly Gly Ile Val
145 150 155 160

Asn Asn Leu Pro Val Glu Pro Phe Gln Glu Ser Gly Ile Pro Thr Val
165 170 175

Cys Val Asp Val Leu Pro Ile Glu Pro Glu Lys Asp Ile Lys Asn Ile
180 185 190

Leu His Ile Leu Leu Arg Ser Phe Phe Leu Ala Val Arg Ser Asn Ser
195 200 205

Glu Lys Arg Lys Glu Phe Cys Asp Leu Val Ile Val Pro Glu Leu Glu
210 215 220

Glu Phe Thr Pro Leu Asp Val Arg Lys Ala Asp Gln Ile Met Glu Arg
225 230 235 240

Gly Tyr Ile Lys Ala Leu Glu Val Leu Ser Glu
245 250

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 297 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Phe Asn Ile Asn Val Phe Val Asn Ile Ser Trp Leu Tyr Phe Ser
1 5 10 15

Gly Ile Val Met Lys Thr Val Glu Glu Tyr Ala Leu Leu Glu Thr Gly
20 25 30

Val Arg Val Phe Tyr Arg Cys Val Ile Pro Glu Lys Ala Phe Asn Thr
35 40 45

Leu Ile Ile Gly Ser His Gly Leu Gly Ala His Ser Gly Ile Tyr Ile
50 55 60

Ser Val Ala Glu Glu Phe Ala Arg His Gly Phe Gly Phe Cys Met His
65 70 75 80

Asp Gln Arg Gly His Gly Arg Thr Ala Ser Asp Arg Glu Arg Gly Tyr
85 90 95

Val Glu Gly Phe His Asn Phe Ile Glu Asp Met Lys Ala Phe Ser Asp
100 105 110

Tyr Ala Lys Trp Arg Val Gly Gly Asp Glu Ile Ile Leu Leu Gly His
115 120 125

Ser Met Gly Gly Leu Ile Ala Leu Leu Thr Val Ala Thr Tyr Lys Glu
130 135 140

Ile Ala Lys Gly Val Ile Ala Leu Ala Pro Ala Leu Gln Ile Pro Leu
145 150 155 160

Thr Pro Ala Arg Arg Leu Val Leu Ser Leu Ala Ser Arg Leu Ala Pro
 165 170 175
 His Ser Lys Ile Thr Leu Gln Arg Arg Leu Pro Gln Lys Pro Glu Gly
 180 185 190
 Phe Gln Arg Ala Lys Asp Ile Glu Tyr Ser Leu Ser Glu Ile Ser Val
 195 200 205
 Lys Leu Val Asp Glu Met Ile Lys Ala Ser Ser Met Phe Trp Thr Ile
 210 215 220
 Ala Gly Glu Ile Asn Thr Pro Val Leu Leu Ile His Gly Glu Lys Asp
 225 230 235 240
 Asn Val Ile Pro Pro Glu Ala Ser Lys Lys Als Tyr Gln Leu Ile Pro
 245 250 255
 Ser Phe Pro Lys Glu Leu Lys Ile Tyr Pro Asp Leu Gly His Asn Leu
 260 265 270
 Phe Phe Glu Pro Gly Ala Val Lys Ile Val Thr Asp Ile Val Glu Trp
 275 280 285
 Val Lys Asn Leu Pro Arg Glu Asn Pro
 290 295

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 262 AMINO ACIDS
 (B) TYPE: AMINO ACID
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Glu Val Tyr Lys Ala Lys Phe Gly Glu Ala Lys Leu Gly Trp Val
 1 5 10 15
 Val Leu Val His Gly Leu Gly Glu His Ser Gly Arg Tyr Gly Arg Leu
 20 25 30
 Ile Lys Glu Leu Asn Tyr Ala Gly Phe Gly Val Tyr Thr Phe Asp Trp
 35 40 45
 Pro Gly His Gly Lys Ser Pro Gly Lys Arg Gly His Thr Ser Val Glu
 50 55 60
 Glu Ala Met Glu Ile Ile Asp Ser Ile Ile Glu Glu Ile Arg Glu Lys
 65 70 75 80
 Pro Phe Leu Phe Gly His Ser Leu Gly Gly Leu Thr Val Ile Arg Tyr
 85 90 95
 Ala Glu Thr Arg Pro Asp Lys Ile Arg Gly Leu Ile Ala Ser Ser Pro
 100 105 110
 Ala Leu Ala Lys Ser Pro Glu Thr Pro Gly Phe Met Val Ala Leu Ala
 115 120 125
 Lys Phe Leu Gly Lys Ile Ala Pro Gly Val Val Leu Ser Asn Gly Ile

Pro Gly Ile Phe Glu Pro Val Glu Tyr Glu Asn Phe Leu Leu Val Asp
 145 150 155 160
 Gly Gly Ile Val Asn Asn Leu Pro Val Glu Pro Leu Glu Lys Phe Lys
 165 170 175
 Glu Pro Ile Ile Gly Val Asp Val Leu Pro Ile Thr Gln Glu Arg Lys
 180 185 190
 Ile Lys Asn Ile Leu His Ile Leu Ile Arg Ser Phe Phe Leu Ala Val
 195 200 205
 Arg Ser Asn Ser Glu Lys Arg Lys Glu Phe Cys Asn Val Val Ile Glu
 210 215 220
 Pro Pro Leu Glu Glu Phe Ser Pro Leu Asp Val Asn Lys Ala Asp Glu
 225 230 235 240
 Ile Phe Cys Gly Asp Met Arg Ala Leu
 245

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 339 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Pro Ala Asn Asp Ser Pro Thr Ile Asp Phe Asn Pro Arg Gly Ile
 1 5 10 15
 Leu Arg Asn Ala His Ala Gln Val Ile Leu Ala Thr Ser Gly Leu Arg
 20 25 30
 Lys Ala Phe Leu Lys Arg Thr His Lys Ser Tyr Leu Ser Thr Ala Gln
 35 40 45
 Trp Leu Glu Leu Asp Ala Gly Asn Gly Val Thr Leu Ala Gly Glu Leu
 50 55 60
 Asn Thr Ala Pro Ala Thr Ala Ser Ser Ser His Pro Ala His Lys Asn
 65 70 75 80
 Thr Leu Val Ile Val Leu His Gly Trp Glu Gly Ser Ser Gln Ser Ala
 85 90 95
 Tyr Ala Thr Ser Ala Gly Ser Thr Leu Phe Asp Asn Gly Phe Asp Thr
 100 105 110
 Phe Arg Leu Asn Phe Arg Asp His Gly Asp Thr Tyr His Leu Asn Arg
 115 120 125
 Gly Ile Phe Asn Ser Ser Leu Ile Asp Glu Val Val Gly Ala Val Lys
 130 135 140
 Ala Ile Gln Gln Gln Thr Asp Tyr Asp Lys Tyr Cys Leu Met Gly Phe
 145 150 155 160
 Ser Leu Gly Gly Asn Phe Ala Leu Arg Val Ala Val Arg Glu Gln His
 165 170 175

Leu Ala Lys Pro Leu Ala Gly Val Leu Ala Val Cys Pro Val Leu Asp
 180 185 190
 Pro Ala His Thr Met Met Ala Leu Asn Arg Gly Ala Phe Phe Tyr Gly
 195 200 205
 Arg Tyr Phe Ala His Lys Trp Lys Arg Ser Leu Thr Ala Lys Leu Ala
 210 215 220 225
 Ala Phe Pro Asp Tyr Lys Tyr Gly Lys Asp Leu Lys Ser Ile His Thr
 230 235 240
 Leu Asp Glu Leu Asn Asn Tyr Phe Ile Pro Arg Tyr Thr Gly Phe Asn
 245 250 255
 Ser Val Ser Glu Tyr Phe Lys Ser Tyr Thr Leu Thr Gly Gln Lys Leu
 260 265 270
 Ala Phe Leu Asn Cys Pro Ser Tyr Ile Leu Ala Ala Gly Asp Asp Pro
 275 280 285
 Ile Ile Pro Ala Ser Asp Phe Gln Lys Ile Ala Lys Pro Ala Asn Leu
 290 295 300 305
 His Ile Thr Val Thr Gln Gln Gly Ser His Cys Ala Tyr Leu Glu Asn
 310 315 320
 Leu His Lys Pro Ser Ala Ala Asp Lys Tyr Ala Val Lys Leu Phe Gly
 325 330 335
 Ala Cys

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS
 (A) LENGTH: 311 AMINO ACIDS
 (B) TYPE: AMINO ACID
 (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Leu Asp Met Pro Ile Asp Pro Val Tyr Tyr Gln Leu Ala Glu Tyr
 1 5 10 15
 Phe Asp Ser Leu Pro Lys Phe Asp Gln Phe Ser Ser Ala Arg Glu Tyr
 20 25 30
 Arg Glu Ala Ile Asn Arg Ile Tyr Glu Glu Arg Asn Arg Gln Leu Ser
 35 40 45
 Gln His Glu Arg Val Glu Arg Val Glu Asp Arg Thr Ile Lys Gly Arg
 50 55 60
 Asn Gly Asp Ile Arg Val Arg Val Tyr Gln Gln Lys Pro Asp Ser Pro
 65 70 75 80
 Val Leu Val Tyr Tyr His Gly Gly Gly Phe Val Ile Cys Ser Ile Glu
 85 90 95
 Ser His Asp Ala Leu Cys Arg Arg Ile Ala Arg Leu Ser Asn Ser Thr
 100 105 110

Val Val Ser Val Asp Tyr Arg Leu Ala Pro Glu His Lys Phe Pro Ala
 115 120 125
 Ala Val Tyr Asp Cys Tyr Aso Ala Thr Lys Trp Val Ala Glu Asn Ala
 130 135 140
 Glu Glu Leu Arg Ile Asp Pro Ser Lys Ile Phe Val Gly Gly Asp Ser
 145 150 155 160
 Ala Gly Gly Asn Leu Ala Ala Ala Val Ser Ile Met Ala Arg Asp Ser
 165 170 175
 Gly Glu Asp Phe Ile Lys His Gln Ile Leu Ile Tyr Pro Val Val Asn
 180 185 190
 Phe Val Ala Pro Thr Pro Ser Leu Leu Glu Phe GLy Glu Gly Leu Trp
 195 200 205
 Ile Leu Asp Gln Lys Ile Met Ser Trp Phe Ser Glu Gln Tyr Phe Ser
 210 215 230
 Arg Glu Glu Aso Lys Phe Asn Pro Leu Ala Ser Val Ile Phe Ala Asp
 235 240 245 250
 Leu Glu Asn Leu Pro Pro Ala Leu Ile Ile Thr Ala Glu Tyr Asp Pro
 255 260 265
 Leu Arg Asp Glu Gly Glu Val Phe Gly Gln Met Leu Arg Arg Ala Gly
 270 275 280
 Val Glu Ala Ser Ile Val Arg Tyr Arg Gly Val Leu His Gly Phe Ile
 285 290 295
 Asn Tyr Tyr Pro Val Leu Lys Ala Ala Arg Asp Ala Ile Asn Gln Ile
 300 305 310
 Ala Ala Leu leu Val Phe Asp
 315 320

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS
 - (A) LENGTH: 305 AMINO ACIDS
 - (B) TYPE: AMINO ACID
 - (D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Pro Leu Asp Pro Arg Ile Lys Lys Leu Leu Glu Ser Ala Leu Thr
 5 10 15
 Ile Pro Ile Gly Lys Ala Pro Val Glu Glu Val Arg Lys Ile Phe Arg
 20 25 30
 Gln Leu Ala Ser Ala Ala Pro Lys Val Glu Val Gly Lys Val Glu Asp
 35 40 45
 Ile Lys Ile Pro Gly Ser Glu Thr Val Ile Asn Ala Arg Val Tyr Phe
 50 55 60

Pro Lys Ser Ser Gly Pro Tyr Glu Val Leu Val Tyr Leu His Gly Gly
 65 70 75 80
 Gly Phe Val Ile Gly Asp Val Glu Ser Tyr Asp Pro Leu Cys Arg Ala
 85 90 95
 Ile Thr Asn Ala Cys Asn Cys Val Val Ser Val Asp Tyr Arg Leu
 100 105 110
 Ala Pro Glu Tyr Lys Phe Pro Ser Ala Val Ile Asp Ser Phe Asp Ala
 115 120 125
 Thr Asn Trp Val Tyr Asn Asn Leu Asp Lys Phe Asp Gly Lys Met Gly
 130 135 140
 Val Ala Ile Ala Gly Asp Ser Ala Gly Gly Asn Leu Ala Ala Val Val
 145 150 155 160
 Ala Leu Leu Ser Lys Gly Lys Ile Asn Leu Lys Tyr Gln Ile Leu Val
 165 170 175
 Tyr Pro Ala Val Ser Leu Asp Asn Val Ser Arg Ser Met Ile Glu Tyr
 180 185 190
 Ser Asp Gly Phe Phe Leu Thr Arg Glu His Ile Glu Trp Phe Gly Ser
 195 200 205
 Gln Tyr Leu Arg Ser Pro Ala Asp Leu Leu Asp Phe Arg Phe Ser Pro
 210 215 220
 Ile Leu Ala Gln Asp Phe Asn Gly Leu Pro Pro Ala Leu Ile Ile Thr
 225 230 235 240
 Ala Glu Tyr Asp Pro Leu Arg Asp Gln Gly Glu Ala Tyr Ala Asn Lys
 245 250 255
 Leu Leu Gln Ala Gly Val Ser Val Thr Ser Val Arg Phe Asn Asn Val
 260 265 270
 Ile His Gly Phe Leu Ser Phe Phe Pro Leu Met Glu Gln Gly Arg Asp
 275 280 285
 Ala Ile Gly Leu Ile Gly Ser Val Leu Arg Arg Val Phe Tyr Asp Lys
 290 295 300
 Ile
 305

What Is Claimed Is:

1. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide having at least a 70% identity to a polynucleotide encoding an enzyme comprising amino acid sequences set forth in SEQ ID NOS:33-42;

(b) a polynucleotide which is complementary to the polynucleotide of (a);
and

(c) a polynucleotide comprising at least 15 consecutive bases of the polynucleotide of (a) or (b).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 414 of SEQ ID NO:33.

5. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 373 of SEQ ID NO:34.

6. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 453 of SEQ ID NO:35.

7. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 343 of SEQ ID NO:36.

8. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 398 of SEQ ID NO:37.

9. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 592 of SEQ ID NO:38.

10. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 354 of SEQ ID NO:39.

11. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 303 of SEQ ID NO:40.

12. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 311 of SEQ ID NO:41.

13. The polynucleotide of Claim 2 which encodes an enzyme comprising amino acids 1 to 305 of SEQ ID NO:42.

14. An isolated polynucleotide comprising a member selected from the group consisting of:

- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding an enzyme expressed by the DNA contained in ATCC Deposit No. _____;
- (b) a polynucleotide complementary to the polynucleotide of (a); and
- (c) a polynucleotide comprising at least 15 consecutive bases of the polynucleotide of (a) and (b).

15. A vector comprising the DNA of Claim 2.

16. A host cell comprising the vector of Claim 15.

17. A process for producing a polypeptide comprising: expressing from the host cell of Claim 16 a polypeptide encoded by said DNA.

18. A process for producing a cell comprising: transforming or transfecting the cell with the vector of Claim 15 such that the cell expresses the polypeptide encoded by the DNA contained in the vector.

19. An enzyme comprising a member selected from the group consisting of an enzyme comprising an amino acid sequence which is at least 70% identical to the amino acid sequence set forth in SEQ ID NOS:33-42.

20. A method for transferring an amino group from an amino acid to an α -keto acid comprising:

contacting an amino acid in the presence of an α -keto acid with an enzyme selected from the group consisting of an enzyme having the amino acid sequence set forth in SEQ ID NOS:33-42.

ABSTRACT

Esterase enzymes derived from various *Staphylothermus*, *Pyrodictium*, *Archaeoglobus*, *Aquifex*, M11TL, *Thermococcus*, *Teredinibacter* and *Sulfolobus* organisms are disclosed. The enzymes are produced from native or recombinant host cells and can be utilized in the pharmaceutical, agricultural and other industries.

FIGURE 1

Staphylothermus marinus - F1-12LC

ATG	TCT	TTA	AAC	AAG	CAC	TCT	TGG	ATG	GAT	ATG	ATA	ATA	TTT	ATT	CTC
Met	Ser	Leu	Asn	Lys	His	Ser	Trp	Met	Asp	Met	Ile	Ile	Phe	Ile	Leu
AGC	TTT	TCT	TTC	CCA	TTA	ACA	ATG	ATC	GCA	TTA	GCT	ATC	TCT	ATG	TCG
Ser	Phe	Ser	Phe	Pro	Leu	Thr	Met	Ile	Ala	Leu	Ala	Ile	Ser	Met	Ser
TCA	TGG	TTT	AAT	ATA	TGG	AAT	AAT	GCA	TTA	AGC	GAT	CTA	GGA	CAT	GCT
Ser	Trp	Phe	Asn	Ile	Trp	Asn	Asn	Ala	Leu	Ser	Asp	Leu	Gly	His	Ala
GTT	AAA	AGC	AGT	GTT	GCT	CCA	ATA	TTC	AAT	CTA	GGT	CTT	GCA	ATT	GGT
Val	Lys	Ser	Ser	Val	Ala	Pro	Ile	Phe	Asn	Leu	Gly	Leu	Ala	Ile	Gly
GGG	ATA	CTA	ATT	GTT	ATA	GTT	GGT	TTA	AGA	AAT	CTT	TAT	TCG	TGG	AGT
Gly	Ile	Leu	Ile	Val	Ile	Val	Gly	Leu	Arg	Asn	Leu	Tyr	Ser	Trp	Ser
AGA	GTT	AAA	GGA	TCT	TTA	ATC	ATA	TCC	ATG	GGT	GTA	TTT	CTT	AAC	TTA
Arg	Val	Lys	Gly	Ser	Leu	Ile	Ile	Ser	Met	Gly	Val	Phe	Leu	Asn	Leu
ATA	GGG	GTT	TTC	GAC	GAA	GTA	TAT	GGT	TGG	ATA	CAT	TTC	CTA	GTC	TCA
Ile	Gly	Val	Phe	Asp	Glu	Val	Tyr	Gly	Trp	Ile	His	Phe	Leu	Val	Ser
GTA	TTG	TTT	TTC	TTA	TCA	ATA	ATA	GCA	TAT	TTC	ATA	GCT	ATA	TCA	ATA
Val	Leu	Phe	Phe	Leu	Ser	Ile	Ile	Ala	Tyr	Phe	Ile	Ala	Ile	Ser	Ile
CTT	GAC	AAA	TCA	TGG	ATA	GCT	GTT	CTA	CTA	ATA	ATA	GGT	CAT	ATT	GCA
Leu	Asp	Lys	Ser	Trp	Ile	Ala	Val	Leu	Leu	Ile	Ile	Gly	His	Ile	Ala
ATG	TGG	TAT	CTA	CAC	TTT	GCT	TCA	GAG	ATT	CCG	AGA	GGT	GCG	GCT	ATT
Met	Trp	Tyr	Leu	His	Phe	Ala	Ser	Glu	Ile	Pro	Arg	Gly	Ala	Ala	Ile
CCC	GAG	TTA	TTA	GCG	GTA	TTC	TCG	TTT	TTA	CCA	TTC	TAT	ATA	AGA	GAC
Pro	Glu	Leu	Leu	Ala	Val	Phe	Ser	Phe	Leu	Pro	Phe	Tyr	Ile	Arg	Asp
TAT	TTT	AAA	TCA	TAC	ACT	AAA	CGA	TAG							
Tyr	Phe	Lys	Ser	Tyr	Thr	Lys	Arg								

FIGURE 2

Pyrodictium - TAG11-17LC

ATG	AAA	CTC	CTT	GAG	CCC	ACA	AAT	ACC	TCC	TAC	ACG	CTG	TTA	CAG	GAT
Met	Lys	Leu	Leu	Glu	Pro	Thr	Asn	Thr	Ser	Tyr	Thr	Leu	Leu	Gln	Asp
TTA	GCA	TTG	CAT	TTT	GCA	TTT	TAC	TGG	TTT	CTG	GCC	GTG	TAT	ACG	TGG
Leu	Ala	Leu	His	Phe	Ala	Phe	Tyr	Trp	Phe	Leu	Ala	Val	Tyr	Thr	Trp
TTA	CCC	GGT	GTC	CTA	GTC	CGG	GGC	GTA	GCT	GTG	GAC	ACA	GGG	GTG	GCT
Leu	Pro	Gly	Val	Leu	Val	Arg	Gly	Val	Ala	Val	Asp	Thr	Gly	Val	Ala
CGG	GTG	CCT	GGG	CTC	GGC	CGG	CGC	GGT	AAG	AGG	CTG	CTC	CTG	GCC	GCT
Arg	Val	Pro	Gly	Leu	Gly	Arg	Arg	Gly	Lys	Arg	Leu	Leu	Leu	Ala	Ala
GTG	GCT	GTC	TTG	GCG	CTT	GTT	GTG	TCC	GTT	GTT	GTC	CCG	GCT	TAT	GTG
Val	Ala	Val	Leu	Ala	Leu	Val	Val	Ser	Val	Val	Val	Pro	Ala	Tyr	Val
GCG	TAT	AGT	AGT	CTG	CAC	CCG	GAG	AGC	TGT	CGG	CCC	GTT	GCG	CCG	GAG
Ala	Tyr	Ser	Ser	Leu	His	Pro	Glu	Ser	Cys	Arg	Pro	Val	Ala	Pro	Glu
GGG	CTC	ACC	TAC	AAA	GAG	TTC	AGC	GTG	ACC	GCG	GAG	GAT	GGC	TTG	GTG
Gly	Leu	Thr	Tyr	Lys	Glu	Phe	Ser	Val	Thr	Ala	Glu	Asp	Gly	Leu	Val
GTT	CGG	GGC	TGG	GTG	CTG	GGC	CCC	GGC	GCT	GGG	GGC	AAC	CCG	GTG	TTC
Val	Arg	Gly	Trp	Val	Leu	Gly	Pro	Gly	Ala	Gly	Gly	Asn	Pro	Val	Phe
GTT	TTG	ATG	CAC	GGG	TAT	ACT	GGG	TGC	CGC	TCG	GCG	CCC	TAC	ATG	GCT
Val	Leu	Met	His	Gly	Tyr	Thr	Gly	Cys	Arg	Ser	Ala	Pro	Tyr	Met	Ala
GTG	CTG	GCC	CGG	GAG	CTC	GTG	GAG	TGG	GGG	TAC	CCG	GTG	GTT	GTG	TTC
Val	Leu	Ala	Arg	Glu	Leu	Val	Glu	Trp	Gly	Tyr	Pro	Val	Val	Val	Phe
GAC	TTC	CGG	GGC	CAC	GGG	GAG	AGC	GGG	GGC	TCG	ACG	ACG	ATT	GGG	CCC
Asp	Phe	Arg	Gly	His	Gly	Glu	Ser	Gly	Gly	Ser	Thr	Thr	Ile	Gly	Pro
CGG	GAG	GTG	CTG	GAT	GCC	CGG	GCT	GTG	GTG	GGC	TAT	GTC	TCG	GAG	CGG
Arg	Glu	Val	Leu	Asp	Ala	Arg	Ala	Val	Val	Gly	Tyr	Val	Ser	Glu	Arg
TTC	CCC	GGC	CGC	CGG	ATA	ATA	TTG	GTG	GGG	TTC	AGT	ATG	GGC	GGC	GCT
Phe	Pro	Gly	Arg	Arg	Ile	Ile	Leu	Val	Gly	Phe	Ser	Met	Gly	Gly	Ala
GTA	GCG	ATC	GTG	GAG	GGT	GCT	GGG	GAC	CCG	CGG	GTC	TAC	GCG	GTG	GCT
Val	Ala	Ile	Val	Glu	Gly	Ala	Gly	Asp	Pro	Arg	Val	Tyr	Ala	Val	Ala
GCT	GAT	AGC	CCG	TAC	TAT	AGG	CTC	CGG	GAC	GTC	ATA	CCC	CGG	TGG	CTG
Ala	Asp	Ser	Pro	Tyr	Tyr	Arg	Leu	Arg	Asp	Val	Ile	Pro	Arg	Trp	Leu
GAG	TAC	AAG	ACG	CCG	CTG	CCG	GGC	TGG	GTG	GGT	GTG	CTG	GCC	GGG	TTC

FIGURE 4

Aquifex pyrophilus - 28LC

TTG	AGA	TTG	AGG	AAA	TTT	GAA	GAG	ATA	AAC	CTC	GTT	CTT	TCG	GGA	GGA
Leu	Arg	Leu	Arg	Lys	Phe	Glu	Glu	Ile	Asn	Leu	Val	Leu	Ser	Gly	Gly
GCT	GCA	AAG	GGC	ATA	GCC	CAC	ATA	GGT	GTT	TTG	AAA	GCT	ATA	AAC	GAG
Ala	Ala	Lys	Gly	Ile	Ala	His	Ile	Gly	Val	Leu	Lys	Ala	Ile	Asn	Glu
CTC	GGT	ATA	AGG	GTG	AGG	GCT	TTA	AGC	GGG	GTG	AGC	GCC	GGG	GCA	ATC
Leu	Gly	Ile	Arg	Val	Arg	Ala	Leu	Ser	Gly	Val	Ser	Ala	Gly	Ala	Ile
GTT	TCG	GTC	TTT	TAT	GCC	TCA	GGC	TAC	TCC	CCT	GAA	GGG	ATG	TTC	AGC
Val	Ser	Val	Phe	Tyr	Ala	Ser	Gly	Tyr	Ser	Pro	Glu	Gly	Met	Phe	Ser
CTT	CTG	AAG	AGG	GTA	AAC	TGG	CTG	AAG	CTG	TTT	AAG	TTC	AAG	CCA	CCT
Leu	Leu	Lys	Arg	Val	Asn	Trp	Leu	Lys	Leu	Phe	Lys	Phe	Lys	Pro	Pro
CTG	AAG	GGA	TTG	ATA	GGG	TGG	GAG	AAG	GCT	ATA	AGA	TTC	CTT	GAG	GAA
Leu	Lys	Gly	Leu	Ile	Gly	Trp	Glu	Lys	Ala	Ile	Arg	Phe	Leu	Glu	Glu
GTT	CTC	CCT	TAC	AGG	AGA	ATA	GAA	AAA	CTT	GAG	ATA	CCG	ACG	TAT	ATA
Val	Leu	Pro	Tyr	Arg	Arg	Ile	Glu	Lys	Leu	Glu	Ile	Pro	Thr	Tyr	Ile
TGC	GCG	ACG	GAT	TTA	TAC	TCG	GGA	AGG	GCT	CTA	TAC	CTC	TCG	GAA	GGG
Cys	Ala	Thr	Asp	Leu	Tyr	Ser	Gly	Arg	Ala	Leu	Tyr	Leu	Ser	Glu	Gly
AGT	TTA	ATC	CCC	GCA	CTT	CTC	GGC	AGC	TGT	GCA	ATT	CCC	GGC	ATA	TTT
Ser	Leu	Ile	Pro	Ala	Leu	Leu	Gly	Ser	Cys	Ala	Ile	Pro	Gly	Ile	Phe
GAA	CCC	GTT	GAG	TAT	AAG	AAT	TAC	TTG	CTC	GTT	GAC	GGA	GGT	ATA	GTT
Glu	Pro	Val	Glu	Tyr	Lys	Asn	Tyr	Leu	Leu	Val	Asp	Gly	Gly	Ile	Val
AAC	AAC	CTT	CCC	GTT	GAG	CCC	TTT	CAG	GAA	AGC	GGT	ATT	CCC	ACC	GTT
Asn	Asn	Leu	Pro	Val	Glu	Pro	Phe	Gln	Glu	Ser	Gly	Ile	Pro	Thr	Val
TGC	GTT	GAT	GTC	CTT	CCC	ATA	GAG	CCG	GAA	AAG	GAT	ATA	AAG	AAC	ATT
Cys	Val	Asp	Val	Leu	Pro	Ile	Glu	Pro	Glu	Lys	Asp	Ile	Lys	Asn	Ile
CTT	CAC	ATC	CTT	TTG	AGG	AGC	TTC	TTT	CTT	GCG	GTC	CGC	TCA	AAC	TCC
Leu	His	Ile	Leu	Leu	Arg	Ser	Phe	Phe	Leu	Ala	Val	Arg	Ser	Asn	Ser
GAA	AAG	AGA	AAG	GAG	TTT	TGT	GAC	CTC	GTT	ATA	GTT	CCT	GAG	CTT	GAG
Glu	Lys	Arg	Lys	Glu	Phe	Cys	Asp	Leu	Val	Ile	Val	Pro	Glu	Leu	Glu
GAG	TTC	ACA	CCC	CTT	GAT	GTT	AGA	AAA	GCG	GAC	CAA	ATA	ATG	GAG	AGG
Glu	Phe	Thr	Pro	Leu	Asp	Val	Arg	Lys	Ala	Asp	Gln	Ile	Met	Glu	Arg

FIGURE 5

M11TL-29L.

ATG	TTT	AAT	ATC	AAT	GTC	TTT	GTT	AAT	ATA	TCT	TGG	CTG	TAT	TTT	TCA
Met	Phe	Asn	Ile	Asn	Val	Phe	Val	Asn	Ile	Ser	Trp	Leu	Tyr	Phe	Ser
GGG	ATA	GTT	ATG	AAG	ACT	GTG	GAA	GAG	TAT	GCG	CTA	CTT	GAA	ACA	GGC
Gly	Ile	Val	Met	Lys	Thr	Val	Glu	Glu	Tyr	Ala	Leu	Leu	Glu	Thr	Gly
GTA	AGA	GTG	TTT	TAT	CGG	TGT	GTA	ATC	CCG	GAG	AAA	GCT	TTT	AAC	ACT
Val	Arg	Val	Phe	Tyr	Arg	Cys	Val	Ile	Pro	Glu	Lys	Ala	Phe	Asn	Thr
TTG	ATA	ATA	GGT	TCA	CAC	GGA	TTG	GGG	GCG	CAC	AGT	GGA	ATC	TAC	ATT
Leu	Ile	Ile	Gly	Ser	His	Gly	Leu	Gly	Ala	His	Ser	Gly	Ile	Tyr	Ile
AGT	GTT	GCT	GAA	GAA	TTT	GCT	AGG	CAC	GGA	TTT	GGA	TTC	TGC	ATG	CAC
Ser	Val	Ala	Glu	Glu	Phe	Ala	Arg	His	Gly	Phe	Gly	Phe	Cys	Met	His
GAT	CAA	AGG	GGA	CAT	GGG	AGA	ACG	GCA	AGC	GAT	AGA	GAA	AGA	GGG	TAT
Asp	Gln	Arg	Gly	His	Gly	Arg	Thr	Ala	Ser	Asp	Arg	Glu	Arg	Gly	Tyr
GTG	GAG	GGC	TTT	CAC	AAC	TTC	ATA	GAG	GAT	ATG	AAG	GCC	TTC	TCC	GAT
Val	Glu	Gly	Phe	His	Asn	Phe	Ile	Glu	Asp	Met	Lys	Ala	Phe	Ser	Asp
TAT	GCC	AAG	TGG	CGC	GTG	GGA	GGT	GAC	GAA	ATA	ATA	TTG	CTA	GGA	CAC
Tyr	Ala	Lys	Trp	Arg	Val	Gly	Gly	Asp	Glu	Ile	Ile	Leu	Leu	Gly	His
AGT	ATG	GGC	GGG	CTG	ATA	GCG	CTC	TTA	ACA	GTT	GCA	ACT	TAT	AAA	GAA
Ser	Met	Gly	Gly	Leu	Ile	Ala	Leu	Leu	Thr	Val	Ala	Thr	Tyr	Lys	Glu
ATC	GCC	AAG	GGA	GTT	ATC	GCG	CTA	GCC	CCG	GCC	CTC	CAA	ATC	CCC	TTA
Ile	Ala	Lys	Gly	Val	Ile	Ala	Leu	Ala	Pro	Ala	Leu	Gln	Ile	Pro	Leu
ACC	CCG	GCT	AGA	AGA	CTT	GTT	CTA	AGC	CTC	GCG	TCA	AGG	CTT	GCC	CCG
Thr	Pro	Ala	Arg	Arg	Leu	Val	Leu	Ser	Leu	Ala	Ser	Arg	Leu	Ala	Pro
CAT	TCT	AAG	ATC	ACC	TTA	CAA	AGG	AGA	TTG	CCG	CAG	AAA	CCA	GAG	GGT
His	Ser	Lys	Ile	Thr	Leu	Gln	Arg	Arg	Leu	Pro	Gln	Lys	Pro	Glu	Gly
TTT	CAA	AGA	GCA	AAA	GAT	ATA	GAA	TAC	AGT	CTG	AGT	GAA	ATA	TCA	GTC
Phe	Gln	Arg	Ala	Lys	Asp	Ile	Glu	Tyr	Ser	Leu	Ser	Glu	Ile	Ser	Val
AAG	CTC	GTG	GAC	GAA	ATG	ATT	AAA	GCA	TCA	TCT	ATG	TTC	TGG	ACC	ATA
Lys	Leu	Val	Asp	Glu	Met	Ile	Lys	Ala	Ser	Ser	Met	Phe	Trp	Thr	Ile
GCA	GGG	GAA	ATT	AAT	ACT	CCC	GTC	CTG	CTT	ATT	CAT	GGG	GAA	AAA	GAC
Ala	Gly	Glu	Ile	Asn	Thr	Pro	Val	Leu	Leu	Ile	His	Gly	Glu	Lys	Asp

FIGURE 6

Thermococcus CL-2-30LC

ATG	GAG	GTT	TAC	AAG	GCC	AAA	TTC	GGC	GAA	GCA	AAG	CTC	GGC	TGG	GTC
Met	Glu	Val	Tyr	Lys	Ala	Lys	Phe	Gly	Glu	Ala	Lys	Leu	Gly	Trp	Val
GTT	CTG	GTT	CAT	GGC	CTC	GGC	GAG	CAC	AGC	GGA	AGG	TAT	GGA	AGA	CTG
Val	Leu	Val	His	Gly	Leu	Gly	Glu	His	Ser	Gly	Arg	Tyr	Gly	Arg	Leu
ATT	AAG	GAA	CTC	AAC	TAT	GCC	GGC	TTT	GGA	GTT	TAC	ACC	TTC	GAC	TGG
Ile	Lys	Glu	Leu	Asn	Tyr	Ala	Gly	Phe	Gly	Val	Tyr	Thr	Phe	Asp	Trp
CCC	GGC	CAC	GGG	AAG	AGC	CCG	GGC	AAG	AGA	GGG	CAC	ACG	AGC	GTC	GAG
Pro	Gly	His	Gly	Lys	Ser	Pro	Gly	Lys	Arg	Gly	His	Thr	Ser	Val	Glu
GAG	GCG	ATG	GAA	ATC	ATC	GAC	TCG	ATA	ATC	GAG	GAG	ATC	AGG	GAG	AAG
Glu	Ala	Met	Glu	Ile	Ile	Asp	Ser	Ile	Ile	Glu	Glu	Ile	Arg	Glu	Lys
CCC	TTC	CTC	TTC	GGC	CAC	AGC	CTC	GGT	GGT	CTA	ACT	GTC	ATC	AGG	TAC
Pro	Phe	Leu	Phe	Gly	His	Ser	Leu	Gly	Gly	Leu	Thr	Val	Ile	Arg	Tyr
GCT	GAG	ACG	CGG	CCC	GAT	AAA	ATA	CGG	GGA	TTA	ATA	GCT	TCC	TCG	CCT
Ala	Glu	Thr	Arg	Pro	Asp	Lys	Ile	Arg	Gly	Leu	Ile	Ala	Ser	Ser	Pro
GCC	CTC	GCC	AAG	AGC	CCG	GAA	ACG	CCG	GGC	TTC	ATG	GTG	GCC	CTC	GCG
Ala	Leu	Ala	Lys	Ser	Pro	Glu	Thr	Pro	Gly	Phe	Met	Val	Ala	Leu	Ala
AAG	TTC	CTT	GGA	AAG	ATC	GCC	CCG	GGA	GTT	GTT	CTC	TCC	AAC	GGC	ATA
Lys	Phe	Leu	Gly	Lys	Ile	Ala	Pro	Gly	Val	Val	Leu	Ser	Asn	Gly	Ile
AAG	CCG	GAA	CTC	CTC	TCG	AGG	AAC	AGG	GAC	GCC	GTG	AGG	AGG	TAC	GTT
Lys	Pro	Glu	Leu	Leu	Ser	Arg	Asn	Arg	Asp	Ala	Val	Arg	Arg	Tyr	Val
GAA	GAC	CCA	CTC	GTC	CAC	GAC	AGG	ATT	TCG	GCC	AAG	CTG	GGA	AGG	AGC
Glu	Asp	Pro	Leu	Val	His	Asp	Arg	Ile	Ser	Ala	Lys	Leu	Gly	Arg	Ser
ATC	TTC	GTG	AAC	ATG	GAG	CTG	GCC	CAC	AGG	GAG	GCG	GAC	AAG	ATA	AAA
Ile	Phe	Val	Asn	Met	Glu	Leu	Ala	His	Arg	Glu	Ala	Asp	Lys	Ile	Lys
GTC	CCG	ATC	CTC	CTT	CTG	ATC	GGC	ACT	GGC	GAT	GTA	ATA	ACC	CCG	CCT
Val	Pro	Ile	Leu	Leu	Leu	Ile	Gly	Thr	Gly	Asp	Val	Ile	Thr	Pro	Pro
GAA	GGC	TCA	CGC	AGA	CTC	TTC	GAG	GAG	CTG	GCC	GTC	GAG	AAC	AAA	ACC
Glu	Gly	Ser	Arg	Arg	Leu	Phe	Glu	Glu	Leu	Ala	Val	Glu	Asn	Lys	Thr
CTG	AGG	GAG	TTC	GAG	GGG	GCG	TAC	CAC	GAG	ATA	TTT	GAA	GAC	CCC	GAG
Leu	Arg	Glu	Phe	Glu	Gly	Ala	Tyr	His	Glu	Ile	Phe	Glu	Asp	Pro	Glu

FIGURE 7

Aquifex VF5-34LC

TTG	ATT	GGC	AAT	TTG	AAA	TTG	AAG	AGG	TTT	GAA	GAG	GTT	AAC	TTA	GTT	
Leu	Ile	Gly	Asn	Leu	Lys	Leu	Lys	Arg	Phe	Glu	Glu	Val	Asn	Leu	Val	
CTT	TCG	GGA	GGG	GCT	GCC	AAG	GGT	ATC	GCC	CAT	ATA	GGT	GTT	TTA	AAA	
Leu	Ser	Gly	Gly	Ala	Ala	Lys	Gly	Ile	Ala	His	Ile	Gly	Val	Leu	Lys	
GCT	CTG	GAA	GAG	CTC	GGT	ATA	AAG	GTA	AAG	AGG	CTC	AGC	GGG	GTA	AGT	
Ala	Leu	Glu	Glu	Leu	Gly	Ile	Lys	Val	Lys	Arg	Leu	Ser	Gly	Val	Ser	
GCT	GGA	GCT	ATC	GTT	TCC	GTC	TTT	TAC	GCT	TCG	GGC	TAC	ACT	CCC	GAC	
Ala	Gly	Ala	Ile	Val	Ser	Val	Phe	Tyr	Ala	Ser	Gly	Tyr	Thr	Pro	Asp	
GAG	ATG	TTA	AAA	CTC	CTG	AAA	GAG	GTA	AAC	TGG	CTC	AAA	CTT	TTT	AAG	
Glu	Met	Leu	Lys	Leu	Leu	Lys	Glu	Val	Asn	Trp	Leu	Lys	Leu	Phe	Lys	
TTC	AAA	ACA	CCG	AAA	ATG	GGC	TTA	ATG	GGG	TGG	GAG	AAG	GCT	GCA	GAG	
Phe	Lys	Thr	Pro	Lys	Met	Gly	Leu	Met	Gly	Trp	Glu	Lys	Ala	Ala	Glu	
TTT	TTG	GAA	AAA	GAG	CTC	GGA	GTT	AAG	AGG	CTG	GAA	GAC	CTG	AAC	ATA	
Phe	Leu	Glu	Lys	Glu	Leu	Gly	Val	Lys	Arg	Leu	Glu	Asp	Leu	Asn	Ile	
CCA	ACC	TAT	CTT	TGC	TCG	GCG	GAT	CTG	TAC	ACG	GGA	AAG	GCT	CTT	TAC	
Pro	Thr	Tyr	Leu	Cys	Ser	Ala	Asp	Leu	Tyr	Thr	Gly	Lys	Ala	Leu	Tyr	
TTC	GGC	AGA	GGT	GAC	TTA	ATT	CCC	GTG	CTT	CTC	GGA	AGT	TGT	TCC	ATA	
Phe	Gly	Arg	Gly	Asp	Leu	Ile	Pro	Val	Leu	Leu	Gly	Ser	Cys	Ser	Ile	
CCC	GGG	ATT	TTT	GAA	CCA	GTT	GAG	TAC	GAG	AAT	TTT	CTA	CTT	GTT	GAC	
Pro	Gly	Ile	Phe	Glu	Pro	Val	Glu	Tyr	Glu	Asn	Phe	Leu	Leu	Val	Asp	
GGA	GGT	ATA	GTG	AAC	AAC	CTG	CCC	GTA	GAA	CCT	TTG	GAA	AAG	TTC	AAA	
Gly	Gly	Ile	Val	Asn	Asn	Leu	Pro	Val	Glu	Pro	Leu	Glu	Lys	Phe	Lys	
GAA	CCC	ATA	ATC	GGG	GTA	GAT	GTG	CTT	CCC	ATA	ACT	CAA	GAA	AGA	AAG	
Glu	Pro	Ile	Ile	Gly	Val	Asp	Val	Leu	Pro	Ile	Thr	Gln	Glu	Arg	Lys	
ATT	AAA	AAT	ATA	CTC	CAC	ATC	CTT	ATA	AGG	AGC	TTC	TTT	CTG	GCG	GTT	
Ile	Lys	Asn	Ile	Leu	His	Ile	Leu	Ile	Arg	Ser	Phe	Phe	Leu	Ala	Val	
CGT	TCC	AAT	TCG	GAA	AAG	AGA	AAG	GAG	TTC	TGC	AAC	GTA	GTT	ATA	GAA	
Arg	Ser	Asn	Ser	Glu	Lys	Arg	Lys	Glu	Phe	Cys	Asn	Val	Val	Ile	Glu	
CCT	CCC	CTT	GAA	GAG	TTC	TCT	CCT	CTG	GAC	GTA	AAT	AAG	GCG	GAC	GAG	
Pro	Pro	Leu	Glu	Glu	Phe	Ser	Pro	Leu	Asp	Val	Asn	Lys	Ala	Asp	Glu	

FIGURE 8

Teredinibacter - 42L

ATG	CCA	GCT	AAT	GAC	TCA	CCC	ACG	ATC	GAC	TTT	AAT	CCT	CGC	GGC	ATT
Met	Pro	Ala	Asn	Asp	Ser	Pro	Thr	Ile	Asp	Phe	Asn	Pro	Arg	Gly	Ile
CTT	CGC	AAC	GCT	CAC	GCA	CAG	GTT	ATT	TTA	GCG	ACT	TCC	GGC	TTG	CGC
Leu	Arg	Asn	Ala	His	Ala	Gln	Val	Ile	Leu	Ala	Thr	Ser	Gly	Leu	Arg
AAA	GCG	TTT	TTG	AAA	CGC	ACG	CAC	AAG	AGC	TAC	CTC	AGC	ACT	GCC	CAA
Lys	Ala	Phe	Leu	Lys	Arg	Thr	His	Lys	Ser	Tyr	Leu	Ser	Thr	Ala	Gln
TGG	CTG	GAG	CTC	GAT	GCC	GGC	AAC	GGA	GTT	ACC	TTG	GCC	GGA	GAG	CTT
Trp	Leu	Glu	Leu	Asp	Ala	Gly	Asn	Gly	Val	Thr	Leu	Ala	Gly	Glu	Leu
AAC	ACA	GCG	CCT	GCA	ACT	GCA	TCC	TCC	TCC	CAC	CCG	GCG	CAC	AAG	AAC
Asn	Thr	Ala	Pro	Ala	Thr	Ala	Ser	Ser	Ser	His	Pro	Ala	His	Lys	Asn
ACT	CTG	GTT	ATT	GTG	CTG	CAC	GGC	TGG	GAA	GGC	TCC	AGC	CAG	TCG	GCC
Thr	Leu	Val	Ile	Val	Leu	His	Gly	Trp	Glu	Gly	Ser	Ser	Gln	Ser	Ala
TAT	GCG	ACC	TCC	GCT	GGC	AGC	ACG	CTT	TTC	GAC	AAT	GGG	TTC	GAC	ACT
Tyr	Ala	Thr	Ser	Ala	Gly	Ser	Thr	Leu	Phe	Asp	Asn	Gly	Phe	Asp	Thr
TTT	CGC	CTT	AAT	TTT	CGC	GAT	CAC	GGC	GAC	ACC	TAC	CAC	TTA	AAC	CGC
Phe	Arg	Leu	Asn	Phe	Arg	Asp	His	Gly	Asp	Thr	Tyr	His	Leu	Asn	Arg
GGC	ATA	TTT	AAC	TCA	TCG	CTG	ATT	GAC	GAA	GTA	GTG	GGC	GCA	GTC	AAA
Gly	Ile	Phe	Asn	Ser	Ser	Leu	Ile	Asp	Glu	Val	Val	Gly	Ala	Val	Lys
GCC	ATC	CAG	CAG	CAA	ACC	GAC	TAC	GAC	AAG	TAT	TGC	CTG	ATG	GGG	TTC
Ala	Ile	Gln	Gln	Gln	Thr	Asp	Tyr	Asp	Lys	Tyr	Cys	Leu	Met	Gly	Phe
TCA	CTG	GGT	GGG	AAC	TTT	GCC	TTG	CGC	GTC	GCG	GTG	CGG	GAA	CAG	CAT
Ser	Leu	Gly	Gly	Asn	Phe	Ala	Leu	Arg	Val	Ala	Val	Arg	Glu	Gln	His
CTC	GCT	AAA	CCG	CTA	GCG	GGC	GTG	CTC	GCC	GTA	TGC	CCG	GTA	CTC	GAC
Leu	Ala	Lys	Pro	Leu	Ala	Gly	Val	Leu	Ala	Val	Cys	Pro	Val	Leu	Asp
CCC	GCA	CAC	ACC	ATG	ATG	GCC	CTA	AAC	CGA	GGT	GCG	TTT	TTC	TAC	GGC
Pro	Ala	His	Thr	Met	Met	Ala	Leu	Asn	Arg	Gly	Ala	Phe	Phe	Tyr	Gly
CGC	TAT	TTT	GCG	CAT	AAA	TGG	AAG	CGC	TCG	TTA	ACC	GCA	AAA	CTT	GCA
Arg	Tyr	Phe	Ala	His	Lys	Trp	Lys	Arg	Ser	Leu	Thr	Ala	Lys	Leu	Ala
GCT	TTC	CCA	GAC	TAC	AAA	TAC	GGC	AAA	GAT	TTA	AAA	TCG	ATA	CAC	ACG
Ala	Phe	Pro	Asp	Tyr	Lys	Tyr	Gly	Lys	Asp	Leu	Lys	Ser	Ile	His	Thr

FIGURE 9

Archeoglobus fulgidus VC16 - 16MC1

ATG	CTT	GAT	ATG	CCA	ATC	GAC	CCT	GTT	TAC	TAC	CAG	CTT	GCT	GAG	TAT
Met	Leu	Asp	Met	Pro	Ile	Asp	Pro	Val	Tyr	Tyr	Gln	Leu	Ala	Glu	Tyr
TTC	GAC	AGT	CTG	CCG	AAG	TTC	GAC	CAG	TTT	TCC	TCG	GCC	AGA	GAG	TAC
Phe	Asp	Ser	Leu	Pro	Lys	Phe	Asp	Gln	Phe	Ser	Ser	Ala	Arg	Glu	Tyr
AGG	GAG	GCG	ATA	AAT	CGA	ATA	TAC	GAG	GAG	AGA	AAC	CGG	CAG	CTG	AGC
Arg	Glu	Ala	Ile	Asn	Arg	Ile	Tyr	Glu	Glu	Arg	Asn	Arg	Gln	Leu	Ser
CAG	CAT	GAG	AGG	GTT	GAA	AGA	GTT	GAG	GAC	AGG	ACG	ATT	AAG	GGG	AGG
Gln	His	Glu	Arg	Val	Glu	Arg	Val	Glu	Asp	Arg	Thr	Ile	Lys	Gly	Arg
AAC	GGA	GAC	ATC	AGA	GTC	AGA	GTT	TAC	CAG	CAG	AAG	CCC	GAT	TCC	CCG
Asn	Gly	Asp	Ile	Arg	Val	Arg	Val	Tyr	Gln	Gln	Lys	Pro	Asp	Ser	Pro
GGT	CTG	GTT	TAC	TAT	CAC	GGT	GGT	GGA	TTT	GTG	ATT	TGC	AGC	ATC	GAG
Val	Leu	Val	Tyr	Tyr	His	Gly	Gly	Gly	Phe	Val	Ile	Cys	Ser	Ile	Glu
TCG	CAC	GAC	GCC	TTA	TGC	AGG	AGA	AYY	GCG	AGA	CTT	TCA	AAC	TCT	ACC
Ser	His	Asp	Ala	Leu	Cys	Arg	Arg	Ile	Ala	Arg	Leu	Ser	Asn	Ser	Thr
GTA	GTC	TCC	GTG	GAT	TAC	AGG	CTC	GCT	CCT	GAG	CAC	AAG	TTT	CCC	CCC
Val	Val	Ser	Val	Asp	Tyr	Arg	Leu	Ala	Pro	Glu	His	Lys	Phe	Pro	Ala
CCA	GTT	TAT	CAT	TGC	TAC	GAT	GCG	ACC	AAG	TGG	GTT	GCT	GAG	AAC	CGG
Ala	Val	Tyr	Asp	Cys	Tyr	Asp	Ala	Thr	Lys	Trp	Val	Ala	Glu	Asn	Ala
GAG	GAG	CTG	AGG	ATT	GAC	CCG	TCA	AAA	ATC	TTC	GTT	GGG	GGG	GAC	AGT
Glu	Glu	Leu	Arg	Ile	Asp	Pro	Ser	Lys	Ile	Phe	Val	Gly	Gly	Asp	Ser
GCG	GGA	CGG	AAT	CTT	GCC	CCG	GCG	CTT	TCA	ATA	ATG	GCG	AGA	GAC	AGC
Ala	Gly	Gly	Asn	Leu	Ala	Ala	Ala	Val	Ser	Ile	Met	Ala	Arg	Asp	Ser
GGA	GAA	GAT	TTC	ATA	AAG	CAT	CAA	ATT	CTA	ACT	TAC	CCC	GTT	GTG	AAC
Gly	Glu	Asp	Phe	Ile	Lys	His	Gln	Ile	Leu	Ile	Tyr	Pro	Val	Val	Asn
TTT	GTA	GCC	CCC	ACA	CCA	TCG	CTT	CTG	GAG	TTT	GGA	GAG	GGG	CTG	TGG
Phe	Val	Ala	Pro	Thr	Pro	Ser	Leu	Leu	Glu	Phe	Gly	Glu	Gly	Leu	Trp
ATT	CTC	GAC	CAG	AAG	ATA	ATG	AGT	TGG	TTC	TCG	GAG	CAG	TAC	TTC	TCC
Ile	Leu	Asp	Gln	Lys	Ile	Met	Ser	Trp	Phe	Ser	Glu	Gln	Tyr	Phe	Ser
AGA	GAG	GAA	GAT	AAG	TTC	AAG	CCC	CTC	GCC	TCC	GTA	ATC	TTT	GCG	GAC
Arg	Glu	Glu	Asp	Lys	Phe	Asn	Pro	Leu	Ala	Ser	Val	Ile	Phe	Ala	Asp

FIGURE 10

Sulfolobus Solfataricus P1 - 8LC1

ATG	CCC	CTA	GAT	CCT	AGA	ATT	AAA	AAG	TTA	CTA	GAA	TCA	GCT	CTT	ACT
Met	Pro	Leu	Asp	Pro	Arg	Ile	Lys	Lys	Leu	Leu	Glu	Ser	Ala	Leu	Thr
ATA	CCA	ATT	GGT	AAA	GCC	CCA	GTA	GAA	GAG	GTA	AGA	AAG	ATA	TTT	AGG
Ile	Pro	Ile	Gly	Lys	Ala	Pro	Val	Glu	Glu	Val	Arg	Lys	Ile	Phe	Arg
CAA	TTA	GCG	TCG	GCA	GCT	CCC	AAA	GTC	GAA	GTT	GGA	AAA	GTA	GAA	GAT
Gln	Leu	Ala	Ser	Ala	Ala	Pro	Lys	Val	Glu	Val	Gly	Lys	Val	Glu	Asp
ATA	AAA	ATA	CCA	GGC	AGT	GAA	ACC	GTT	ATA	AAC	GCT	AGA	GTG	TAT	TTT
Ile	Lys	Ile	Pro	Gly	Ser	Glu	Thr	Val	Ile	Asn	Ala	Arg	Val	Tyr	Phe
CCG	AAG	AGT	AGC	GGT	CCT	TAT	GGT	GTT	CTA	GTG	TAT	CTT	CAT	GGA	GGC
Pro	Lys	Ser	Ser	Gly	Pro	Tyr	Gly	Val	Leu	Val	Tyr	Leu	His	Gly	Gly
GGT	TTT	GTA	ATA	GGC	GAT	GTG	GAA	TCT	TAT	GAC	CCA	TTA	TGT	AGA	GCA
Gly	Phe	Val	Ile	Gly	Asp	Val	Glu	Ser	Tyr	Asp	Pro	Leu	Cys	Arg	Ala
ATT	ACA	AAT	GCG	TGC	AAT	TGC	GTT	GTA	GTA	TCA	GTG	GAC	TAT	AGG	TTA
Ile	Thr	Asn	Ala	Cys	Asn	Cys	Val	Val	Val	Ser	Val	Asp	Tyr	Arg	Leu
GCT	CCA	GAA	TAC	AAG	TTT	CCT	TCT	GCA	GTT	ATC	GAT	TCA	TTT	GAC	GCT
Ala	Pro	Glu	Tyr	Lys	Phe	Pro	Ser	Ala	Val	Ile	Asp	Ser	Phe	Asp	Ala
ACT	AAT	TGG	GTT	TAT	AAC	AAT	TTA	GAT	AAA	TTT	GAT	GGA	AAG	ATG	GGA
Thr	Asn	Trp	Val	Tyr	Asn	Asn	Leu	Asp	Lys	Phe	Asp	Gly	Lys	Met	Gly
GTT	GCG	ATT	GCG	GGA	GAT	AGT	GCT	GGA	GGA	AAT	TTG	GCA	GCG	GTT	GTA
Val	Ala	Ile	Ala	Gly	Asp	Ser	Ala	Gly	Gly	Asn	Leu	Ala	Ala	Val	Val
GCT	CTT	CTT	TCA	AAG	GGT	AAA	ATT	AAT	TTG	AAG	TAT	CAA	ATA	CTG	GTT
Ala	Leu	Leu	Ser	Lys	Gly	Lys	Ile	Asn	Leu	Lys	Tyr	Gln	Ile	Leu	Val
TAC	CCA	GCG	GTA	AGT	TTA	GAT	AAC	GTT	TCA	AGA	TCC	ATG	ATA	GAG	TAC
Tyr	Pro	Ala	Val	Ser	Leu	Asp	Asn	Val	Ser	Arg	Ser	Met	Ile	Glu	Tyr
TCT	GAT	GGG	TTC	TTC	CTT	ACC	AGA	GAG	CAT	ATA	GAG	TGG	TTC	GGT	TCT
Ser	Asp	Gly	Phe	Phe	Leu	Thr	Arg	Glu	His	Ile	Glu	Trp	Phe	Gly	Ser
CAA	TAC	TTA	CGA	AGC	CCT	GCA	GAT	TTG	CTA	GAC	TTT	AGG	TTC	TCT	CCA
Gln	Tyr	Leu	Arg	Ser	Pro	Ala	Asp	Leu	Leu	Asp	Phe	Arg	Phe	Ser	Pro

Figure 11
LA11.1 Esterase es2

ATG	AAG	GTT	AAA	CAC	GTT	ATT	GTT	TTA	CAT	GGC	TTA	TAT	ATG	TCT	GGC
Met	Lys	Val	Lys	His	Val	Ile	Val	Leu	His	Gly	Leu	Tyr	Met	Ser	Gly
TTG	GTG	ATG	CGC	CCG	TTA	TGT	TCG	CGT	CTA	GAA	GAG	TCG	GGG	GTT	AAA
Leu	Val	Met	Arg	Pro	Leu	Cys	Ser	Arg	Leu	Glu	Glu	Ser	Gly	Val	Lys
GTT	TTA	AAC	TTA	ACC	TAC	AAT	ACT	CGA	GAC	CCT	AAT	CGA	GAT	GCT	ATT
Val	Leu	Asn	Leu	Thr	Tyr	Asn	Thr	Arg	Asp	Pro	Asn	Arg	Asp	Ala	Ile
TTT	ACG	CAA	ATA	GAT	GAG	TTT	ATT	AGC	AAT	GAG	CCT	TCT	GCT	TTA	GTG
Phe	Thr	Gln	Ile	Asp	Glu	Phe	Ile	Ser	Asn	Glu	Pro	Ser	Ala	Leu	Val
TGT	CAC	TCT	ATG	GGG	GGC	TTA	GTT	GCT	CGC	GCC	TAT	TTA	GAG	GCA	AAC
Cys	His	Ser	Met	Gly	Gly	Leu	Val	Ala	Arg	Ala	Tyr	Leu	Glu	Ala	Asn
TCA	GCG	CCA	AGT	CAT	CAT	GTT	GAA	AAG	GTA	ATC	ACC	TTA	GGA	ACG	CCA
Ser	Ala	Pro	Ser	His	His	Val	Glu	Lys	Val	Ile	Thr	Leu	Gly	Thr	Pro
CAT	ACT	GGC	AGC	CAT	ATT	GCT	GAA	AAA	ATG	CAG	CAA	AAA	GGG	TTC	GAG
His	Thr	Gly	Ser	His	Ile	Ala	Glu	Lys	Met	Gln	Gln	Lys	Gly	Phe	Glu
CTA	TTA	TTA	AAA	AAT	AGC	GTT	GAG	TTT	TTA	CTC	TCT	AAG	AAT	GGT	GAT
Leu	Leu	Leu	Lys	Asn	Ser	Val	Glu	Phe	Leu	Leu	Ser	Lys	Asn	Gly	Asp
TGG	CCT	TTT	AAA	GCC	AAG	CTA	TAT	AGC	ATT	GCC	GGC	GAC	TTA	CCG	ATT
Trp	Pro	Phe	Lys	Ala	Lys	Leu	Tyr	Ser	Ile	Ala	Gly	Asp	Leu	Pro	Ile
GGC	TTA	ATG	CCA	CTC	ATT	GTA	AAA	GGC	AGC	CGC	TCT	GAT	GGC	ACT	GTA
Gly	Leu	Met	Pro	Leu	Ile	Val	Lys	Gly	Ser	Arg	Ser	Asp	Gly	Thr	Val
TTG	CTA	GAT	GAA	ACC	AAG	CTA	AAG	GGT	ATG	GCT	GAA	CAC	AAG	GTG	TTT
Leu	Leu	Asp	Glu	Thr	Lys	Leu	Lys	Gly	Met	Ala	Glu	His	Lys	Val	Phe
CAT	TTA	AGC	CAT	ACA	AGT	ATG	ATT	TAC	TCT	CGC	CAA	GTC	GTT	AAT	TAT
His	Leu	Ser	His	Thr	Ser	Met	Ile	Tyr	Ser	Arg	Gln	Val	Val	Asn	Tyr
ATT	CTT	GAG	CGC	TTG	AAC	GAG	GAC	ATT	TA						
Ile	Leu	Glu	Arg	Leu	Asn	Glu	Asp	Ile							

[illegible]

Figure 13
Metallosphaera Prunae Ron 12/2 Esterase 23mc1

ATG	CCC	CTA	CAT	CCA	AAG	GTA	AAG	AAA	TTA	CTT	TCC	CAG	CTA	CCT	CCC
Met	Pro	Leu	His	Pro	Lys	Val	Lys	Lys	Leu	Leu	Ser	Gln	Leu	Pro	Pro
CAG	GAC	TTC	TCC	AGA	AAC	GTG	CAG	GAC	CTG	AGG	AAG	GCC	TGG	GAT	TTA
Gln	Asp	Phe	Ser	Arg	Asn	Val	Gln	Asp	Leu	Arg	Lys	Ala	Trp	Asp	Leu
CCC	TTC	TCA	GGG	AGG	AGG	GAG	ACC	CTG	AAG	AGG	GTT	GAG	GAC	CTT	GAG
Pro	Phe	Ser	Gly	Arg	Arg	Glu	Thr	Leu	Lys	Arg	Val	Glu	Asp	Leu	Glu
ATA	CCC	ACT	AGG	GAC	GCA	CGA	ATC	AGG	GCC	AGG	GTC	TAC	ACC	CCC	TCA
Ile	Pro	Thr	Arg	Asp	Ala	Arg	Ile	Arg	Ala	Arg	Val	Tyr	Thr	Pro	Ser
AGT	AAG	GAA	AAC	TTA	CCC	GTC	CTT	GTT	TAC	TAT	CAC	GGC	GGT	GGC	TTC
Ser	Lys	Glu	Asn	Leu	Pro	Val	Leu	Val	Tyr	Tyr	His	Gly	Gly	Gly	Phe
GTG	TTC	GGT	AGC	GTT	GAC	AGC	TAC	GAC	GGC	CTC	GCA	TCC	CTT	ATT	GCC
Val	Phe	Gly	Ser	Val	Asp	Ser	Tyr	Asp	Gly	Leu	Ala	Ser	Leu	Ile	Ala
AAG	GAA	TCT	GGG	ATT	GCG	GTT	ATC	TCC	GTG	GAG	TAT	AGG	CTC	GCC	CCT
Lys	Glu	Ser	Gly	Ile	Ala	Val	Ile	Ser	Val	Glu	Tyr	Arg	Leu	Ala	Pro
GAG	CAC	AAG	TTC	CCC	ACC	GCA	GTC	AAC	GAC	TCG	TGG	GAT	GCG	CTT	CTC
Glu	His	Lys	Phe	Pro	Thr	Ala	Val	Asn	Asp	Ser	Trp	Asp	Ala	Leu	Leu
TGG	ATC	GCG	GAG	AAC	GGA	GGC	AAG	CTG	GGG	CTC	GAC	ACC	TCG	AGA	CTT
Trp	Ile	Ala	Glu	Asn	Gly	Gly	Lys	Leu	Gly	Leu	Asp	Thr	Ser	Arg	Leu
GCC	GTG	GCT	GGG	GAT	AGT	GCT	GGA	GGA	AAC	CTG	TCT	GCC	GTG	GTG	TCC
Ala	Val	Ala	Gly	Asp	Ser	Ala	Gly	Gly	Asn	Leu	Ser	Ala	Val	Val	Ser
CTC	CTG	GAC	AGG	GAC	CAG	GGT	AAG	GGA	CTG	GTT	AGT	TAT	CAG	GTC	CTA
Leu	Leu	Asp	Arg	Asp	Gln	Gly	Lys	Gly	Leu	Val	Ser	Tyr	Gln	Val	Leu
ATC	TAC	CCA	GCA	GTG	AAC	ATG	GTC	GAT	AAC	TCC	CCA	TCC	GTC	AGG	GAG
Ile	Tyr	Pro	Ala	Val	Asn	Met	Val	Asp	Asn	Ser	Pro	Ser	Val	Arg	Glu
TAC	GGC	GAG	GGA	TAC	TTC	CTC	ACC	AGG	TCC	ATG	ATG	AAC	TGG	TTC	GGG
Tyr	Gly	Glu	Gly	Tyr	Phe	Leu	Thr	Arg	Ser	Met	Met	Asn	Trp	Phe	Gly
ACC	ATG	TAC	TTC	TCC	TCT	GGA	AGG	GAA	GCG	GTA	TCC	CCC	TAC	GCC	TCT
Thr	Met	Tyr	Phe	Ser	Ser	Gly	Arg	Glu	Ala	Val	Ser	Pro	Tyr	Ala	Ser
CCA	GCC	TTG	GCT	GAC	CTA	CAT	AAC	CTC	CCA	CCC	TCA	CTG	GTG	ATC	ACT
Pro	Ala	Leu	Ala	Asp	Leu	His	Asn	Leu	Pro	Pro	Ser	Leu	Val	Ile	Thr
GCA	GAG	TAT	GAT	CCC	CTA	AGG	GAT	CAG	GGA	GAG	ACC	TAC	TCT	CAC	TCC
Ala	Glu	Tyr	Asp	Pro	Leu	Arg	Asp	Gln	Gly	Glu	Thr	Tyr	Ser	His	Ser

CTA	AAC	GAG	GCT	GGA	AAC	GTA	TCA	ACC	TTG	GTT	AGA	TAT	CAA	GGA	ATG
Leu	Asn	Glu	Ala	Gly	Asn	Val	Ser	Thr	Leu	Val	Arg	Tyr	Gln	Gly	Met
ATT	CAC	GGC	TTC	CTG	TCC	TTC	TAC	GAG	TGG	ATA	ACT	GCC	GGT	AAA	CTA
Ile	His	Gly	Phe	Leu	Ser	Phe	Tyr	Glu	Trp	Ile	Thr	Ala	Gly	Lys	Leu
GCC	ATT	CAC	CAC	ATT	GCT	GGG	GTT	CTG	AGA	TCT	GTC	CTT	TA		
Ala	Ile	His	His	Ile	Ala	Gly	Val	Leu	Arg	Ser	Val	Leu			

Figure 14
Thermotoga neapolitana 5068 Esterase 56mc4

GTG GCC TTC TTC GAT ATG CCC CTT GAG GAA CTG AAA AAG TAC CGG CCT
 Val Ala Phe Phe Asp Met Pro Leu Glu Glu Leu Lys Lys Tyr Arg Pro
 GAA AGG TAC GAG GAG AAA GAT TTC GAT GAG TTC TGG AGG GAA ACA CTT
 Glu Arg Tyr Glu Glu Lys Asp Phe Asp Glu Phe Trp Arg Glu Thr Leu
 AAA GAA AGC GAA GGA TTC CCT CTG GAT CCC GTC TTT GAA AAG GTG GAC
 Lys Glu Ser Glu Gly Phe Pro Leu Asp Pro Val Phe Glu Lys Val Asp
 TTT CAT CTC AAA ACG GTT GAA ACG TAC GAT GTT ACT TTC TCT GGA TAC
 Phe His Leu Lys Thr Val Glu Thr Tyr Asp Val Thr Phe Ser Gly Tyr
 AGG GGG CAG AGA ATA AAG GGC TGG CTT CTT GTT CCG AAG TTG GCG GAA
 Arg Gly Gln Arg Ile Lys Gly Trp Leu Leu Val Pro Lys Leu Ala Glu
 GAA AAG CTT CCA TGC GTC GTG CAG TAC ATA GGT TAC AAT GGT GGA AGG
 Glu Lys Leu Pro Cys Val Val Gln Tyr Ile Gly Tyr Asn Gly Gly Arg
 GGT TTT CCA CAC GAC TGG CTG TTC TGG CCG TCA ATG GGT TAC ATC TGT
 Gly Phe Pro His Asp Trp Leu Phe Trp Pro Ser Met Gly Tyr Ile Cys
 TTT GTC ATG GAC ACC AGG GGG CAG GGA AGC GGC TGG ATG AAG GGA GAC
 Phe Val Met Asp Thr Arg Gly Gln Gly Ser Gly Trp Met Lys Gly Asp
 ACA CCG GAT TAC CCT GAG GGT CCA GTC GAT CCA CAG TAC CCC GGA TTC
 Thr Pro Asp Tyr Pro Glu Gly Pro Val Asp Pro Gln Tyr Pro Gly Phe
 ATG ACG AGG GGC ATT CTG GAT CCG GGA ACC TAT TAC TAC AGG CGA GTC
 Met Thr Arg Gly Ile Leu Asp Pro Gly Thr Tyr Tyr Tyr Arg Arg Val
 TTC GTG GAT GCG GTC AGG GCG GTG GAA GCA GCC ATT TCC TTC CCG AGA
 Phe Val Asp Ala Val Arg Ala Val Glu Ala Ala Ile Ser Phe Pro Arg
 GTG GAT TCC AGG AAG GTG GTG GTG GCC GGA GGC AGT CAG GGT GGG GGA
 Val Asp Ser Arg Lys Val Val Val Ala Gly Gly Ser Gln Gly Gly Gly
 ATC CCC CTT GCG GTG AGT GCC CTG TCG AAC AGG GTG AAG GCT CTG CTC
 Ile Pro Leu Ala Val Ser Ala Leu Ser Asn Arg Val Lys Ala Leu Leu
 TGC GAT GTG CCG TTT CTG TGC CAC TTC AGA AGG GCC GTG CAA CTT GTC
 Cys Asp Val Pro Phe Leu Cys His Phe Arg Arg Ala Val Gln Leu Val
 GAC ACA CAC CCA TAC GTG GAG ATC ACC AAC TTC CTC AAA ACC CAC AGG
 Asp Thr His Pro Tyr Val Glu Ile Thr Asn Phe Leu Lys Thr His Arg
 GAC AAA GAG GAG ATT GTT TTC AGA ACA CTT TCC TAC TTC GAT GGT GTG
 Asp Lys Glu Glu Ile Val Phe Arg Thr Leu Ser Tyr Phe Asp Gly Val

AAC TTT GCA GCA AGG GCA AAG GTG CCC GCC CTG TTT TCC GTT GGG CTC
 Asn Phe Ala Ala Arg Ala Lys Val Pro Ala Leu Phe Ser Val Gly Leu
 ATG GAC ACC ATC TGT CCT CCC TCG ACG GTC TTC GCC GCT TAC AAC CAC
 Met Asp Thr Ile Cys Pro Pro Ser Thr Val Phe Ala Ala Tyr Asn His
 TAC GCC GGT CCA AAG GAG ATC AGA ATC TAT CCG TAC AAC AAC CAC GAA
 Tyr Ala Gly Pro Lys Glu Ile Arg Ile Tyr Pro Tyr Asn Asn His Glu
 GGT GGA GGT TCT TTC CAG GCA ATT GAG CAG GTG AAA TTC TTG AAG AGA
 Gly Gly Gly Ser Phe Gln Ala Ile Glu Gln Val Lys Phe Leu Lys Arg
 CTA TTT GAG GAA GGC TAG

[illegible]

Figure 15
Melittangium lichenicola Esterase 77mc1

ATG CGC ACC CTC TCC TTC GGT CCG ATG ACC ACA GGG GGA AGC ATT CAC
Met Arg Thr Leu Ser Phe Gly Pro Met Thr Thr Gly Gly Ser Ile His
ATG GCG ACC ATG GAC GTG ATG CGC GGG CCG GGG ATG CAG CGG CTG TCA
Met Ala Thr Met Asp Val Met Arg Gly Pro Gly Met Gln Arg Leu Ser
CAG GGC GCC AGG GAG GCC GCG AAC CAC CCC TGG GCG AAG CGA CTG GGC
Gln Gly Ala Arg Glu Ala Ala Asn His Pro Trp Ala Lys Arg Leu Gly
CGC ATG GGC TAC GCG GCC AAG GGC GCC GTG TAC GCC ATC ATC GGC GTG
Arg Met Gly Tyr Ala Ala Lys Gly Ala Val Tyr Ala Ile Ile Gly Val
CTC GCG CTG AAG CTC GCG GCG GGC GAG GGC GGC CGG ACC ACG GAC AGC
Leu Ala Leu Lys Leu Ala Ala Gly Glu Gly Gly Arg Thr Thr Asp Ser
CAC GGC GCG GTG AAC ACC GTG GCG CAC GGG CCC TTC GGC GTC GCG CTG
His Gly Ala Val Asn Thr Val Ala His Gly Pro Phe Gly Val Ala Leu
CTG GCG GTG CTG GTG GTG GGC CTG CTG GGC TAC GTG GTC TGG AGG TTC
Leu Ala Val Leu Val Val Gly Leu Leu Gly Tyr Val Val Trp Arg Phe
GCC CAG GCC TTC GTG GAC ACG GAG GAC AAG GGC TCC GAC GCG AAG GGA
Ala Gln Ala Phe Val Asp Thr Glu Asp Lys Gly Ser Asp Ala Lys Gly
ATC GCC ACG CGC GCC ATG TAC TTC CTC AGC GGC TGC ATC TAC GCG TCG
Ile Ala Thr Arg Ala Met Tyr Phe Leu Ser Gly Cys Ile Tyr Ala Ser
CTG GCC TTC TTC GCC GCG CAG TCC CTG GTG GGC GCC GCG CAC GGC CGG
Leu Ala Phe Phe Ala Ala Gln Ser Leu Val Gly Ala Ala His Gly Arg
AGC AAG GGG ACG CAG GGC TGG ACG GCC ACG CTG ATG GAG CAG CCC TTT
Ser Lys Gly Thr Gln Gly Trp Thr Ala Thr Leu Met Glu Gln Pro Phe
GGC CGC GTG CTG GTG GCG CTG GTG GGG CTG GGC ATC GTG GGC TTC GCG
Gly Arg Val Leu Val Ala Leu Val Gly Leu Gly Ile Val Gly Phe Ala
CTG AAG CAG TTC CAC ACC GCG TGG AAG GCG AAG TTC CGG GAG AAG CTC
Leu Lys Gln Phe His Thr Ala Trp Lys Ala Lys Phe Arg Glu Lys Leu
ACC CTC ACC GGA CTG GCT GCC CGG AAG CAG CAC CAC ATC GAG CGC ATG
Thr Leu Thr Gly Leu Ala Ala Arg Lys Gln His His Ile Glu Arg Met
TGC CAG TTC GGC ATC GCC GCG CGC GGC GTG GTG TTC GCC GTC ATC GGC
Cys Gln Phe Gly Ile Ala Ala Arg Gly Val Val Phe Ala Val Ile Gly
GGC TTC CTC GTC CGC TCC GCC GTG GAC GCG AAC CCC GGC GAG GCC AAG
Gly Phe Leu Val Arg Ser Ala Val Asp Ala Asn Pro Gly Glu Ala Lys

GGC CTG GGA GAG GCC CTG GCC GTC GTC GCG AGG CAG CCG TCC GGC GAC
Gly Leu Gly Glu Ala Leu Ala Val Val Ala Arg Gln Pro Ser Gly Asp

GTG CTC CTG GGG GTG GTG GCG GCG GGC CTG GTG GCC TAC GCC GCC TAC
Val Leu Leu Gly Val Val Ala Ala Gly Leu Val Ala Tyr Ala Ala Tyr
CTG TTC CTC CAG GCG CGC TAC CGC GAA CTC TAG
Leu Phe Leu Gln Ala Arg Tyr Arg Glu Leu

Figure 16

27/33

GAT	CAA	CCC	CAA	GCC	TGC	ATT	GTC	ACC	TGT	GGG	TTT	GAC	CCT	GCG	CGA
Asp	Gln	Pro	Gln	Ala	Cys	Ile	Val	Thr	Cys	Gly	Phe	Asp	Pro	Ala	Arg
CGA	CGG	GAA	CAC	CTA	CGC	CGA	ACG	CTT	AAT	TGC	CGA	GGG	GAT	AGA	CGT
Arg	Arg	Glu	His	Leu	Arg	Arg	Thr	Leu	Asn	Cys	Arg	Gly	Asp	Arg	Arg

TA

Figure 17
Whale Mat Sample AD3059 Esterase es4

GTG AGC ATT CGT CTG CGA CTG TTA AAC TGG TTT TTG AAT ACC TTT GAA
Val Ser Ile Arg Leu Arg Leu Leu Asn Trp Phe Leu Asn Thr Phe Glu
AAA CCA AAA CTG GCC GCG GCC AAA ACG CCG GAT GAT TTG CGA AAA TCG
Lys Pro Lys Leu Ala Ala Ala Lys Thr Pro Asp Asp Leu Arg Lys Ser
TTT GAA TTA AAG GCG AGG TTT TTG TTT CCG GCG CCA CGT AAA ACA AGG
Phe Glu Leu Lys Ala Arg Phe Leu Phe Pro Ala Pro Arg Lys Thr Arg
TTT AGT CAT GAT GTA TTG CAG TCA GGC ATC GGG TCG GTA AAT GCC CAG
Phe Ser His Asp Val Leu Gln Ser Gly Ile Gly Ser Val Asn Ala Gln
TGG GCG AAA TCC AAA TCT GCA TCT GAT GAC AGG GTA ATC CTG TAT TTT
Trp Ala Lys Ser Lys Ser Ala Ser Asp Asp Arg Val Ile Leu Tyr Phe
CAT GGG GGA GGG TAT GTT TTT GGG TCA CCA AAA ACG CAC CGT GCA ATG
His Gly Gly Gly Tyr Val Phe Gly Ser Pro Lys Thr His Arg Ala Met
TTG GCG CGC TTG TCG GCA ATG ACA GGT CTT TCT GCG TGC CTT CCA GAT
Leu Ala Arg Leu Ser Ala Met Thr Gly Leu Ser Ala Cys Leu Pro Asp
TAT AGG TTG GCA CCA GAG CAC CCA TTT CCA GCC GCG ATC GAA GAT GCA
Tyr Arg Leu Ala Pro Glu His Pro Phe Pro Ala Ala Ile Glu Asp Ala
GTT TTA TCG TAT AAA TGT TTA CTA GAG CGA GCA ATC GAG CCC CAA AAT
Val Leu Ser Tyr Lys Cys Leu Leu Glu Arg Ala Ile Glu Pro Gln Asn
ATT ATA CTG GGG GGG GAC AGT GCT GGT GGC GGT TTG GTT CTT GCT TTG
Ile Ile Leu Gly Gly Asp Ser Ala Gly Gly Gly Leu Val Leu Ala Leu
CTT GCA GAA ATC AAG GCC CAA TCC TTG CCC AAA CCT GCT GGC GTT TTT
Leu Ala Glu Ile Lys Ala Gln Ser Leu Pro Lys Pro Ala Gly Val Phe
GCC TTG TCG CCT TTG GTT GAT TTA TCA TTT TCG GGC CTT TCG TTT TCT
Ala Leu Ser Pro Leu Val Asp Leu Ser Phe Ser Gly Leu Ser Phe Ser
AAA AAT GCC CAA ACC GAT GTG ATG TTG CCC GCA TCA CGG GCT GCG GAT
Lys Asn Ala Gln Thr Asp Val Met Leu Pro Ala Ser Arg Ala Ala Asp
ATG GCG ACC TTG TAT TTG GAT GGG GCC GAT GCA GAT GAT CCA CGT GCA
Met Ala Thr Leu Tyr Leu Asp Gly Ala Asp Ala Asp Asp Pro Arg Ala
TCG CCG CTG CAG GCG GAT TTT TCT GGC ATG CCG CCT GTA TTT CTG ACA
Ser Pro Leu Gln Ala Asp Phe Ser Gly Met Pro Pro Val Phe Leu Thr
GCA AGT GAC AGT GAA ATC CTG TTG GAT GAT TGC CTG CGG ATG GCG GAT
Ala Ser Asp Ser Glu Ile Leu Leu Asp Asp Cys Leu Arg Met Ala Asp

CAC TTG CGT GCG CAA GGT GTC GTT GTG ACA GAC CGG ATT GTT GAA AAC
His Leu Arg Ala Gln Gly Val Val Val Thr Asp Arg Ile Val Glu Asn

CAT CCA CAT GTT TGG CAT ATT TTT CAA CGC CTT CTA CCC GAA GCA GAT
His Pro His Val Trp His Ile Phe Gln Arg Leu Leu Pro Glu Ala Asp
CAG GGG CTG CGG GCG ATT GCC GCG TGG ATT AAA CCT CTT TTA TCA GGT
Gln Gly Leu Arg Ala Ile Ala Ala Trp Ile Lys Pro Leu Leu Ser Gly
TCA AAC GAA AGC TA
Ser Asn Glu Ser

Figure 18
***Microscilla furvescens* Esterase 53sc2**

ATG	CTT	ACA	TTT	AAT	GTT	TTA	TAT	GGT	ATG	ATG	AAA	CAA	AAA	CTA	GCA
Met	Leu	Thr	Phe	Asn	Val	Leu	Tyr	Gly	Met	Met	Lys	Gln	Lys	Leu	Ala
GCA	ATT	CTC	ATG	TTT	TTA	GGG	CTA	TCA	GCA	GCA	GAG	GCT	CAA	GAC	TGG
Ala	Ile	Leu	Met	Phe	Leu	Gly	Leu	Ser	Ala	Ala	Glu	Ala	Gln	Asp	Trp
CCT	GAC	CTA	CAG	AAA	TAT	CGT	AGT	GCT	AAT	AAA	GAA	GCC	AAA	TTA	CTT
Pro	Asp	Leu	Gln	Lys	Tyr	Arg	Ser	Ala	Asn	Lys	Glu	Ala	Lys	Leu	Leu
CCA	AAG	GAA	AAC	CGG	AAG	GTG	GTT	TTT	ATG	GGC	AAC	TCC	ATT	ACA	GAA
Pro	Lys	Glu	Asn	Arg	Lys	Val	Val	Phe	Met	Gly	Asn	Ser	Ile	Thr	Glu
GCC	TGG	ATT	AGT	CAG	CGA	CCT	GAG	TTT	TTT	AGT	GAA	AAT	GGG	TTT	ATC
Ala	Trp	Ile	Ser	Gln	Arg	Pro	Glu	Phe	Phe	Ser	Glu	Asn	Gly	Phe	Ile
GGT	CGA	GGC	ATC	AGT	GGC	CAG	ACA	ACC	CCT	CAG	ATG	TTG	TTG	AGA	TTC
Gly	Arg	Gly	Ile	Ser	Gly	Gln	Thr	Thr	Pro	Gln	Met	Leu	Leu	Arg	Phe
CGA	CAG	GAT	GTG	ATA	GAC	CTG	CAG	CCA	AAG	GCT	GTA	GTG	ATA	CTA	GCT
Arg	Gln	Asp	Val	Ile	Asp	Leu	Gln	Pro	Lys	Ala	Val	Val	Ile	Leu	Ala
GGT	ACC	AAT	GAC	GTA	GCT	CAA	AAT	ACC	GGG	CCG	ATG	ACC	ATT	GAG	GAA
Gly	Thr	Asn	Asp	Val	Ala	Gln	Asn	Thr	Gly	Pro	Met	Thr	Ile	Glu	Glu
TCG	CTT	GCT	AAC	ATT	AAG	TCT	ATG	GTG	GAG	CTG	GCG	CAA	GCC	AAT	GGG
Ser	Leu	Ala	Asn	Ile	Lys	Ser	Met	Val	Glu	Leu	Ala	Gln	Ala	Asn	Gly
ATC	ACG	CCT	GTT	TTG	TGT	ACC	GTG	CTG	CCT	GCA	GAT	CGT	TTC	AGC	TGG
Ile	Thr	Pro	Val	Leu	Cys	Thr	Val	Leu	Pro	Ala	Asp	Arg	Phe	Ser	Trp
CGA	CCT	GAG	CTT	ACA	CCC	GCA	GAA	ACT	ATC	ATT	GCC	CTC	AAT	CAG	CTC
Arg	Pro	Glu	Leu	Thr	Pro	Ala	Glu	Thr	Ile	Ile	Ala	Leu	Asn	Gln	Leu
ATT	AAG	CAA	TAT	GCC	GAG	GCA	CAG	GGC	CTG	GCC	CTG	GTG	GAT	TAT	CAT
Ile	Lys	Gln	Tyr	Ala	Glu	Ala	Gln	Gly	Leu	Ala	Leu	Val	Asp	Tyr	His
GCT	GCA	CTC	ACC	AAT	AAA	GGT	GGA	GGA	CTT	CCG	GTG	AAA	TAC	GGA	GAA
Ala	Ala	Leu	Thr	Asn	Lys	Gly	Gly	Gly	Leu	Pro	Val	Lys	Tyr	Gly	Glu
GAT	GGT	GTG	CAT	CCA	AAT	GTA	GCA	GGC	TAT	CAG	GTG	ATG	GAA	AAC	ATT
Asp	Gly	Val	His	Pro	Asn	Val	Ala	Gly	Tyr	Gln	Val	Met	Glu	Asn	Ile
GTT	TTA	CCG	GTC	ATT	TCC	AGC	GAG	TTG	GCA	AAG	CTG	AAG	TA		
Val	Leu	Pro	Val	Ile	Ser	Ser	Glu	Leu	Ala	Lys	Leu	Lys			

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Figure 20
Polyangium brachysporum Esterase 78mc1

TTG	AAG	TAC	TTC	AAA	GCC	CGG	CTT	GCC	GGC	ATC	ACC	TTG	CTC	GGC	CTG
Leu	Lys	Tyr	Phe	Lys	Ala	Arg	Leu	Ala	Gly	Ile	Thr	Leu	Leu	Gly	Leu
CTG	GCC	TGC	ACC	TCG	GCC	TCG	GCG	CAG	ACC	GAG	CCC	ATC	GTG	TTC	GTG
Leu	Ala	Cys	Thr	Ser	Ala	Ser	Ala	Gln	Thr	Glu	Pro	Ile	Val	Phe	Val
CAC	GGC	TAT	TCC	GGC	AGC	GCA	TCC	AAC	TGG	GAC	ACC	ATG	CTG	GGC	CGC
His	Gly	Tyr	Ser	Gly	Ser	Ala	Ser	Asn	Trp	Asp	Thr	Met	Leu	Gly	Arg
TTC	CGG	TCG	AAC	GGT	TAT	GCG	TCC	GGC	TCG	CTC	TAC	ACC	TTC	AAC	TAC
Phe	Arg	Ser	Asn	Gly	Tyr	Ala	Ser	Gly	Ser	Leu	Tyr	Thr	Phe	Asn	Tyr
AAC	TCG	TTG	GTC	AGC	AGC	AAC	CGC	ACC	AGC	GCC	AGC	GAG	CTG	CGC	AGC
Asn	Ser	Leu	Val	Ser	Ser	Asn	Arg	Thr	Ser	Ala	Ser	Glu	Leu	Arg	Ser
TTC	GTC	AAC	ACC	GTG	CGT	TCG	CGC	CAC	GGC	AAC	GCC	CGC	ATC	GCG	CTG
Phe	Val	Asn	Thr	Val	Arg	Ser	Arg	His	Gly	Asn	Ala	Arg	Ile	Ala	Leu
GTC	GCC	CAC	TCC	AAC	GGC	GGG	CTG	GTG	TCG	CGC	TGG	TAT	CGC	GCG	GAG
Val	Ala	His	Ser	Asn	Gly	Gly	Leu	Val	Ser	Arg	Trp	Tyr	Arg	Ala	Glu
CTG	GGC	GGC	GAA	ACG	GCC	ACC	CGC	CGC	TTC	GTG	ACG	CTG	GGC	ACG	CCG
Leu	Gly	Gly	Glu	Thr	Ala	Thr	Arg	Arg	Phe	Val	Thr	Leu	Gly	Thr	Pro
CAC	CGG	GGC	ACC	ACC	TGG	GCC	TAT	GCG	TGC	TAC	AGC	CCC	GCA	TGT	TTC
His	Arg	Gly	Thr	Thr	Trp	Ala	Tyr	Ala	Cys	Tyr	Ser	Pro	Ala	Cys	Phe
GAG	ATG	CGC	CCC	GGC	TCC	AGC	TTG	CTG	ACC	ACG	CTG	GGC	TCG	CGT	GCC
Glu	Met	Arg	Pro	Gly	Ser	Ser	Leu	Leu	Thr	Thr	Leu	Gly	Ser	Arg	Ala
TGC	GAC	CGC	TCG	CTG	TGG	TCG	AAC	ACC	GAC	GGC	ATC	ATC	CTG	CCG	GCG
Cys	Asp	Arg	Ser	Leu	Trp	Ser	Asn	Thr	Asp	Gly	Ile	Ile	Leu	Pro	Ala
TCC	AGC	GCG	CAG	TGT	GGT	GTC	AGC	ACG	CGC	ACT	GCC	GAC	GTC	AGC	CAT
Ser	Ser	Ala	Gln	Cys	Gly	Val	Ser	Thr	Arg	Thr	Ala	Asp	Val	Ser	His
CTC	GAC	CTG	CTG	ACC	GAC	TCT	CGC	GTG	TAC	ACG	CAG	TTG	CGC	ACG	CAG
Leu	Asp	Leu	Leu	Thr	Asp	Ser	Arg	Val	Tyr	Thr	Gln	Leu	Arg	Thr	Gln
TTG	CAA	TGA	GGG	TGA	CGG	TGC	ACC	GAA	CGT	GCA	CCT	G			
Leu	Gln	End	Gly	End	Arg	Cys	Thr	Glu	Arg	Ala	Pro				

6611660-01000000

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ESTERASES

the specification of which [] is attached hereto or [X] was filed on February 16, 1996 as Application Serial No. 08/602,359 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s):

Priority Claimed

Yes No

☐ ☐

(Number)

(Country)

(Day/Month/Year Filed)

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	<u>Pending</u> (Status - patented, pending, abandoned)
_____	_____	_____
(Application Serial No.)	(Filing Date)	(Status - patented, pending, abandoned)
_____	_____	_____

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: John N. Bain (Reg. No. 18,651); John G. Gilfillan, III (Reg. No. 22,746); Elliot M. Olstein (Reg. No. 24,025); Raymond J. Lillie (Reg. No. 31,778); Charles J. Herron (Reg. No. 28,019); William Squire (Reg. No. 25,378); Kenneth S. Weitzman (Reg. No. 36,306); and Gregory Ferraro (Reg. No. 36,134). Address correspondence and telephone calls to Charles J. Herron c/o Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 - (201) 994-1700.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of inventor: Dan E. Robertson

Inventor's signature: Dan E. Robertson

Date: May 8, 1996

Residence: 33 Evergreen Lane, Haddonfield, NJ 08033

Citizenship: United States

Post Office Address: same

Full name of inventor: Dennis Murphy

Inventor's signature: Dennis J. Murphy

Date: May 8, 1996

Residence: 10 Fairway Road, Paoli, PA 19301

Citizenship: United States

Post Office Address: same

Full name of inventor: John Reid

Inventor's signature: John C. Reid

Date: May 8, 1996

Residence: 922 Montgomery Avenue Apt. J-2, Bryn Mawr, PA 19010

Citizenship: United States

Post Office Address: same

Full name of inventor: Anthony M. Maffia
Inventor's signature: [Signature] Date: 5/5/96
Residence: 2505-3A Cedar Tree Drive, Wilmington, DE 19810 Citizenship: United States
Post Office Address: same

Full name of inventor: Steven Link
Inventor's signature: [Signature] Date: 5/8/96
Residence: 108 Gibson Avenue, Wilmington, DE 19803 Citizenship: United States
Post Office Address: same

Full name of inventor: Ronald V. Swanson
Inventor's signature: [Signature] Date: 5/8/96
Residence: 309 N. Lemon Street, Apt. A, Media, PA 19063 Citizenship: United States
Post Office Address: same

Full name of inventor: Patrick V. Warren
Inventor's signature: [Signature] Date: 5/8/96
Residence: 35507 Sheffield Ave., Philadelphia, PA 19163 Citizenship: United States
Post Office Address: same

Full name of inventor: Anna Kosmotka
Inventor's signature: [Signature] Date: 5/8/96
Residence: 224 Harrison Road, Brookhaven, PA 19015 Citizenship: United States
Post Office Address: same

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:) Group Art Unit: (Unassigned)
Robertson et al.)
) Examiner: (Unassigned)
Filed: Herewith)
)
Parent Serial No.: 08/602,359)
)
Parent Filing Date: February 16, 1996)
)
For: ESTERASES)
)
)
)

Assistant Commissioner
for Patents
Washington, D.C. 20231

APPOINTMENT OF ASSOCIATE ATTORNEY

Sir:

I am attorney of record in the above-referenced patent application and, pursuant to
37 C.F.R. 1.34b, I hereby appoint:

TIM ELLIS
ROBROY R. FAWCETT
LISA A. HAILE
WILLIAM N. HULSEY III,
RICHARD J. IMBRA
DAVID F. KLEINSMITH
JUNE M. LEARN
TIMOTHY W. LOHSE
TERRANCE A. MEADOR
JOHN OSKOREP
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MIKE SHEVLIN
STEVEN R. SPRINKLE
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BARRY N. YOUNG

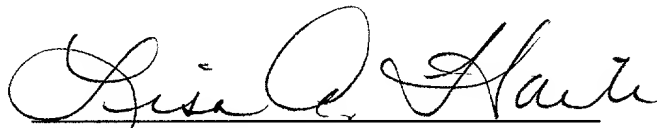
Registration No. 41,734
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Registration No. 40,825
Registration No. 38,322
Registration No. 38,626
Registration No. 27,774

In re Application of:
Robertson et al.
Application No.: Unassigned
Filed: Herewith
Page 2

as associate attorney of record to prosecute this application as well as any continuation and divisional applications and to transact all business in the Patent and Trademark Office in connection therewith.

Respectfully submitted,

Date: August 24, 1999



Lisa A. Haile, Ph.D.

Telephone: (858) 677-1456

Facsimile: (858) 677-1465

GRAY, CARY, WARE & FREIDENRICH, LLP
4365 Executive Drive, Suite 1600
San Diego, California 92121-2189